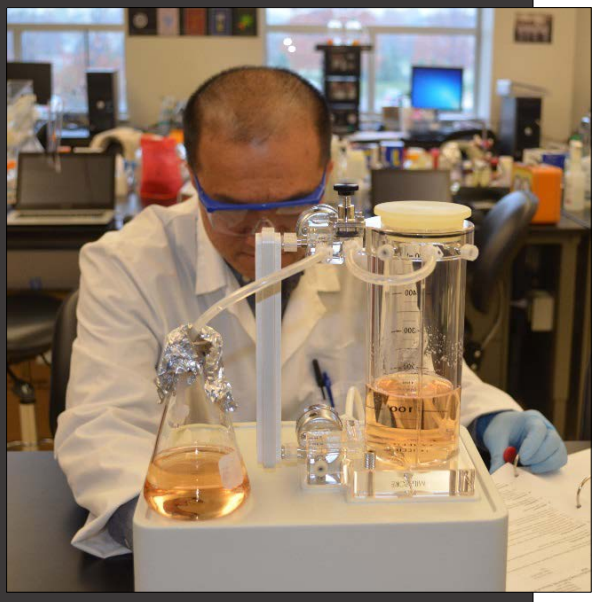
MONTGOMERY COUNTY COMMUNITY COLLEGE
BIOMANUFACTURING LABORATORY MANUAL
2nd Edition



Developed by:

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METROLOGY

SOP: Oakton PC 700 Bench Series pH/ Conductivity/°C/°F Meter

Approvals:

Preparer: Jason McMillan Date 08JAN14
Reviewer: Dr. Margaret Bryans Date 10JAN14

1. **Purpose:**
   1.1. To calibrate and operate the Oakton PC 700 Bench Series pH/ Conductivity/°C/°F Meter.
2. **Scope:**
   2.1. To measure the pH and conductivity of solutions and media.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Oakton Bench 700 Series Quick Guide
   4.2. Oakton PC 700 Instruction Manual
5. **Definitions:** N/A
6. **Precautions:**
   6.1. Use caution when handling all samples due to unknown pH.
   6.2. Do not wipe or rub pH electrode. This will create a static build up that will interfere with measurements.
   6.3. Always wear the appropriate personal protective equipment (PPE).
7. **Materials:**
   7.1. Oakton PC 700 pH Meter and electrodes
   7.2. pH electrode storage solution
   7.3. commercially made pH standard buffers as required
   7.4. wash bottle
   7.5. MilliQ water
   7.6. waste beaker
   7.7. laboratory tissues, such as Kimwipes
8. **Procedure:**
   8.1. **pH Preparation**
      8.1.1. Select the pH 7 standard buffer and a second standard (and third if needed) buffer that brackets the expected sample pH.
      8.1.2. Prepare buffers according to manufacturer's instructions if needed, and ensure that they are not expired.
   8.2. **pH Calibration**
      8.2.1. Press the power key to turn on the pH meter.
      8.2.2. Gently remove the protective cap/sleeve from the bottom of the pH electrode.
      8.2.3. Rinse the pH electrode with MilliQ water and gently blot dry with a laboratory tissue.
      8.2.4. Press the "MODE" key until "pH" is displayed on the right side of primary (top)

reading.

8.2.5. Dip the pH electrode into the first pH buffer and press the "CAL/MEAS" key. The secondary (bottom) reading will lock onto the appropriate buffer value. Provide stirring for best results. When the **READY** indicator appears in the top left portion of the LCD screen, press the "ENTER" key to accept. The primary reading will flash briefly before the secondary reading begins scrolling the remaining buffers available.

8.2.6. Rinse the pH electrode with MilliQ water, gently blot dry with a laboratory tissue, and then dip the pH electrode into the second pH buffer. The secondary reading will lock onto the appropriate buffer value. When the READY indicator appears, press the "ENTER" key to accept. The primary reading will flash briefly and then display the percent efficiency (slope) before the secondary reading begins scrolling the remaining available buffers.

8.2.7. Press the "CAL/MEAS" key to return to measurement mode or to calibrate a third buffer repeat step 8.2.6.

8.2.8. Remove the pH electrode from the solution, rinse the pH electrode with MilliQ water and blot dry with a laboratory tissue.

8.2.9. Replace storage cap if immediate measurement of sample is not needed.

8.3. **pH Measurement**

8.3.1. If necessary, gently remove the protective cap/sleeve from the bottom of the pH electrode, rinse the pH electrode with MilliQ water and blot dry with laboratory tissue. Be sure **MEAS** is visible on the top left side of the LCD display

8.3.2. Insert pH electrode into the sample. Provide stirring.

8.3.3. Read pH value on the primary reading when the value has stabilized.

8.3.4. Remove the pH electrode from the solution, rinse the pH electrode with MilliQ water and blot dry with a laboratory tissue.

8.3.5. Repeat steps 8.3.2. through 8.3.4. for additional samples.

8.3.6. When finished, replace protective cap onto end of the pH electrode and turn off the pH meter by pressing the power key.

8.4. **Automatic Conductivity Calibration**

8.4.1. Press the "MODE" key as needed to select conductivity (µS or mS).

8.4.2. Dip the conductivity electrode into the selected conductivity standard beyond the upper steel band (utilize the fill line on the outside of the probe guard for reference) and press the "CAL/MEAS" key. Provide stirring.

8.4.3. The primary reading will show the factory default value, while the secondary reading will lock on the appropriate automatic standard value from Table 1.

8.4.4. When the **READY** indicator appears, press the "ENTER/RANGE" key to accept. The primary reading will flash briefly before returning to measurement mode upon successful calibration.

8.5. **Conductivity Measurement**

8.5.1. Rinse the conductivity electrode with MilliQ water and gently blot dry with a laboratory tissue.

SOP: Oakton PC 700 Bench Series pH/ Conductivity/°C/°F Meter

8.5.2. Dip the conductivity electrode into the sample beyond the upper steel band (utilize the fill line on the outside of the probe guard for reference).

8.5.3. Allow time for the reading to stabilize on the primary reading. **The clear yellow protective probe guard must be attached during measurement to prevent erroneous results.**

8.5.4. Rinse the conductivity electrode with MilliQ water and gently shake off any excess water droplets.

8.5.5. Turn off the pH/Conductivity meter by pressing the power key.

9. Attachments:

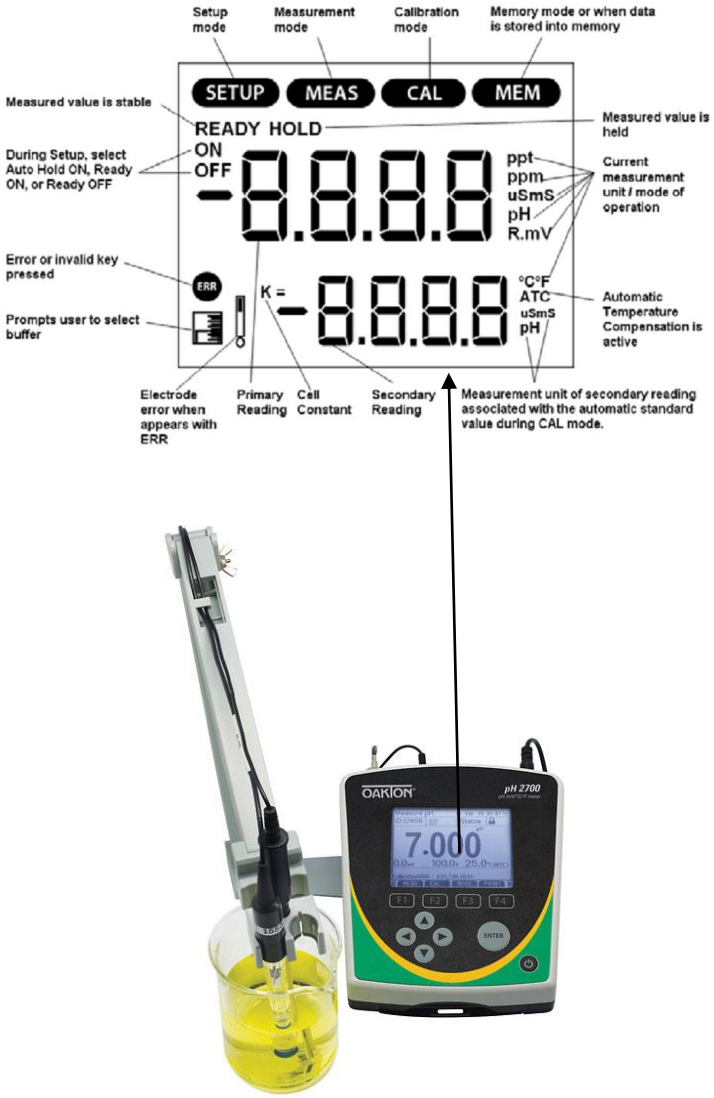
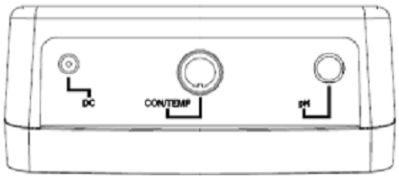


Figure 1: Oakton PC 700 LCD Annunciators
(http://static.coleparmer.com/large_images/35420_20a.jpg)
Oakton PC 700 Manual

SOP: Oakton PC 700 Bench Series pH/ Conductivity/°C/°F Meter



DC	Power supply
CON/ TEMP	8-pin DIN connection for 2-cell Con/TDS/Temp electrode
pH	BNC connection for pH, or ORP (Redox) electrode

Figure 2: Meter Connections
Oakton PC 700 Manual

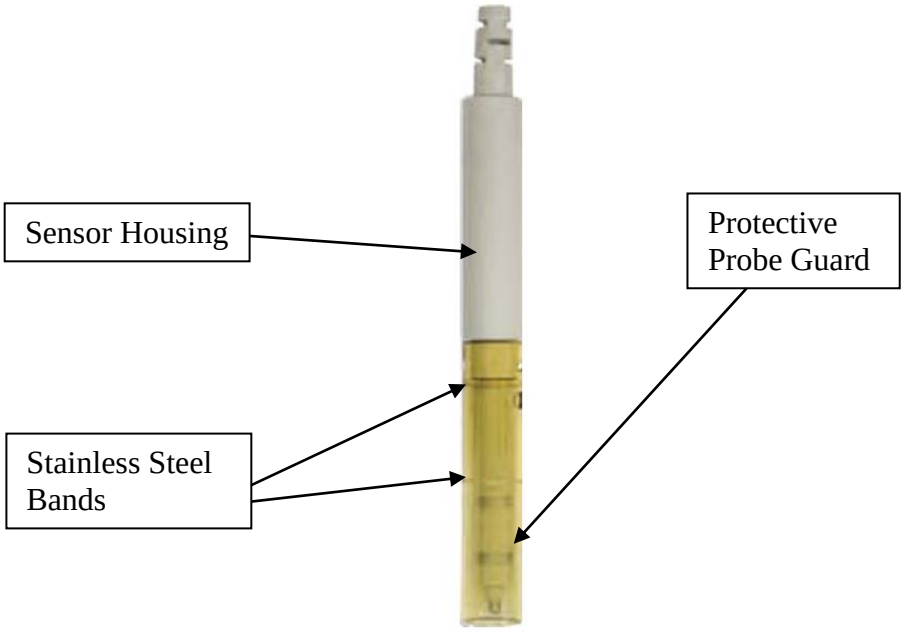


Figure 3: Conductivity Electrode
Oakton PC 700 Manual

SOP: Oakton PC 700 Bench Series pH/ Conductivity/°C/°F Meter

Range #	Conductivity Range	Automatic Calibration Values	
		Normalization Temperature	
		25 °C	20 °C
r 1	0.00 – 20.00 µS	None	None
r 2	20.1 – 200.0 µS	84 µS	76 µS
r 3	201 – 2000 µS	1413 µS	1278 µS
r 4	2.01 – 20.00 mS	12.88 mS	11.67 mS
r 5	20.1 – 200.0 mS	111.8 mS	102.1 mS

Range #	TDS Range (using 0.5 TDS factor)	Automatic Calibration Values
r 1	0 – 10.00 ppm	none
r 2	10.1 – 100.0 ppm	none
r 3	101 – 1000 ppm	none
r 4	1.01 – 10.00 ppt	none
r 5	10.1 – 100 ppt	none

Table 1: Conductivity Calibration Standard values
Oakton PC 700 Manual

10. History:

Revision Number	*Effective Date*	*Preparer*	*Description of Change*
0	08JAN14	Jason McMillan	Initial release

SOP: Denver Instruments APX 323 Balance Calibration

Approvals:

Preparer: Jason McMillan — Date: 10JAN14
Reviewer: Tim Kull — Date: 11JAN14
Reviewer: Dr. Maggie Bryans — Date: 12JAN14

1. **Purpose:**
   1.1. Calibration of Denver Instruments APX 323 Balance.
2. **Scope:**
   2.1. Applies to the calibration of Denver Instruments APX 323 Balance.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Denver Instruments APX 323 Balance Instruction Manual
   4.2. Denver Instruments APX 323 Balance Operation SOP
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. 200g ASTM Weight Class 2 Mass Standards for External Calibration
   7.2. Denver Instruments APX 323 Balance
8. **Procedure:**
   8.1. **Calibration**
      8.1.1. Press “I/O” button to turn the balance on.
      8.1.2. Be sure the weighing pan is clear and press the “ZERO” button to tare the balance.
      8.1.3. Press and hold the “PRINT/MENU” button for three seconds or until the word **Unit** appears on the screen and then release.
      8.1.4. Press the “PRINT/MENU” button and **Cal** is displayed.
      8.1.5. Press the “ARROW” button to enter calibration mode.
      8.1.6. The proper weight needed for calibration is then displayed in grams, 200g in this case.
      8.1.7. Place the 200g ASTM Class 2 Mass Standard on the weighing pan. **Use utensils, do not handle mass standard with your hands**.
      8.1.8. When a stable reading has been recorded, the balance will beep, the screen will flash (- -) and the reading will return to the weight of the calibration weight.
      8.1.9. Calibration is complete. Remove the 200g ASTM Class 2 Mass Standard and the balance is now ready to use.

SOP: Denver Instruments APX 323 Balance Calibration

8. **Attachments:**

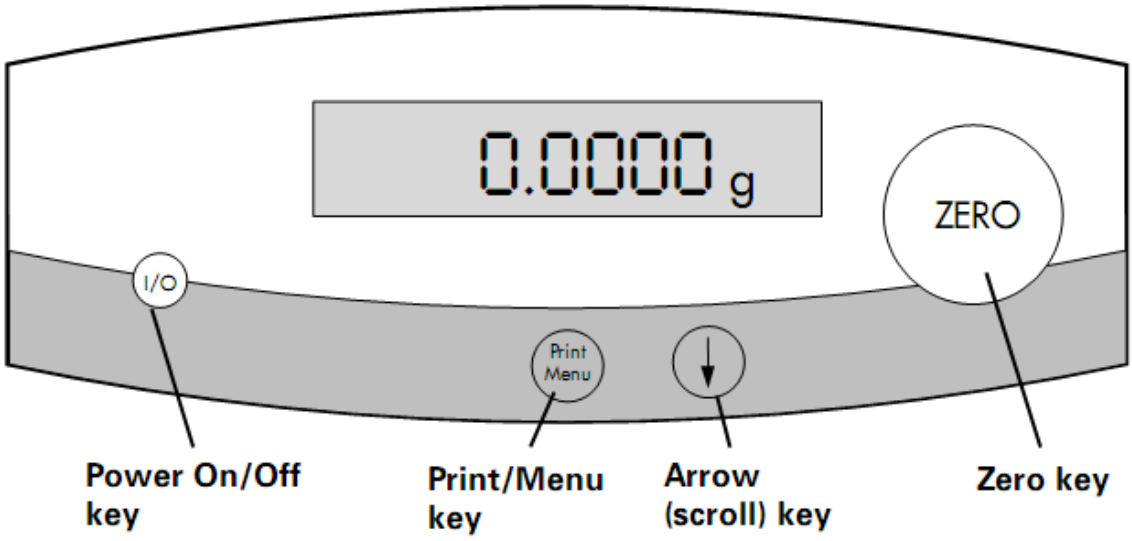


Figure 1: Denver Instrument APX 323 Display and Keyboard
Denver Instrument APX 323 Manual

10. History:

Revision Number	*Effective Date*	*Preparer*	*Description of Change*
0	10JAN14	Jason McMillan	Initial release

SOP: Denver Instrument APX 323 Balance Operation

Approvals:

Preparer: Jason McMillan Date: 10JAN14
Reviewer: Dr. Maggie Bryans Date: 11JAN14

1. **Purpose:**
   1.1. Operation of Denver Instrument APX 323 Balance.
2. **Scope:**
   2.1. Applies to the weight measurement of laboratory materials.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Denver Instrument APX 323 Balance Instruction Manual.
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. Appropriate weighing container(s) such as a weigh boat, weigh paper, beaker, flask, bottle, etc.
8. **Procedure:**
   8.1. **Simple Weighing**
      8.1.1. Turn the scale on using the "I/O" button.
      8.1.2. Place weighing container on the balance weighing pan. Press the "ZERO" button to zero the weight display. (- -) will appear on the display indicating the balance is being zeroed. The balance will then read 0.0000 after successful taring.
      8.1.3. Remove the weighing container. Load weighing container with material to be weighed. Place loaded weighing container on the weighing pan.
      8.1.4. When the reading is stable the unit of measurement will appear in the bottom right-hand corner.
      8.1.5. Record the weight and remove the loaded weighing container.
      8.1.6. To turn balance off, press and hold the "ON/OFF" button.

   8.2. Select Weighing Units
      8.2.1. Press "ON/OFF" button to turn the balance on.
      8.2.2. Press the "Arrow KEY" button to scroll between units.

SOP: Denver Instrument APX 323 Balance Operation

9. Attachments:

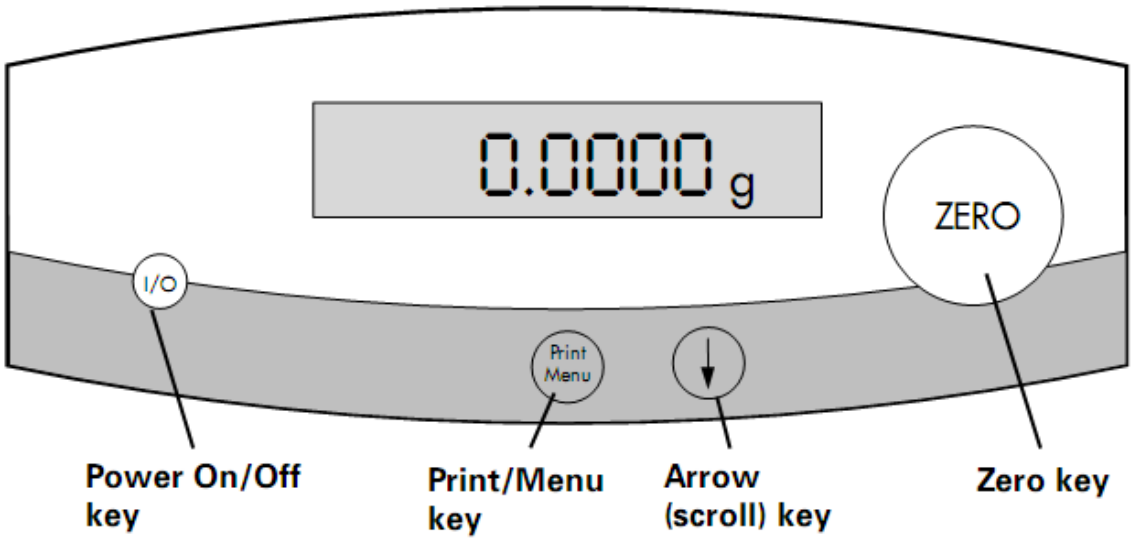


Figure 1: Denver Instrument APX 323 Display and Keyboard
Denver Instrument APX 323 Manual

10. History:

Revision Number	*Effective Date*	*Preparer*	*Description of Change*
0	10JAN14	Jason McMillan	Initial release

SOP: Denver Instruments PI 403 Balance Calibration

Approvals:

Preparer: Jason McMillan Date: 09JAN14
Reviewer: Dr. Maggie Bryans Date: 10JAN14

1. **Purpose:**
   1.1. Calibration of Denver Instruments PI 403 Balance.
2. **Scope:**
   2.1. Applies to the calibration of Denver Instruments PI 403 Balance.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Denver Instruments PI 403 Balance Instruction Manual
   4.2. Denver Instruments PI 403 Balance Operation SOP
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. 200g Class 2 or better mass standard for External Calibration
   7.2. Denver Instruments SI 4002 Balance
8. **Procedure:**
   8.1. **External Calibration**
      8.1.1. Press the "TARE" button.
      8.1.2. Place the 200g Class 2 or better mass standard on the weighing pan. **Use utensils do not touch the mass standard with your hands.**
      8.1.3. Press the "CALIBRATION" button.
      8.1.4. The unit will display **CAL external**.
      8.1.5. When calibration is complete the reading of the weight will be displayed and the unit will return to measurement mode.
   8.2. **Internal Calibration**
      8.3.1. Press the "TARE" button.
      8.3.2. Press the "CALIBRATION" button.
      8.3.3. The unit will display **CAL internal** and perform the calibration.
      8.3.4. When calibration is complete, the unit will return to measurement mode.

SOP: Denver Instruments PI 403 Balance Calibration

9. **Attachments:**

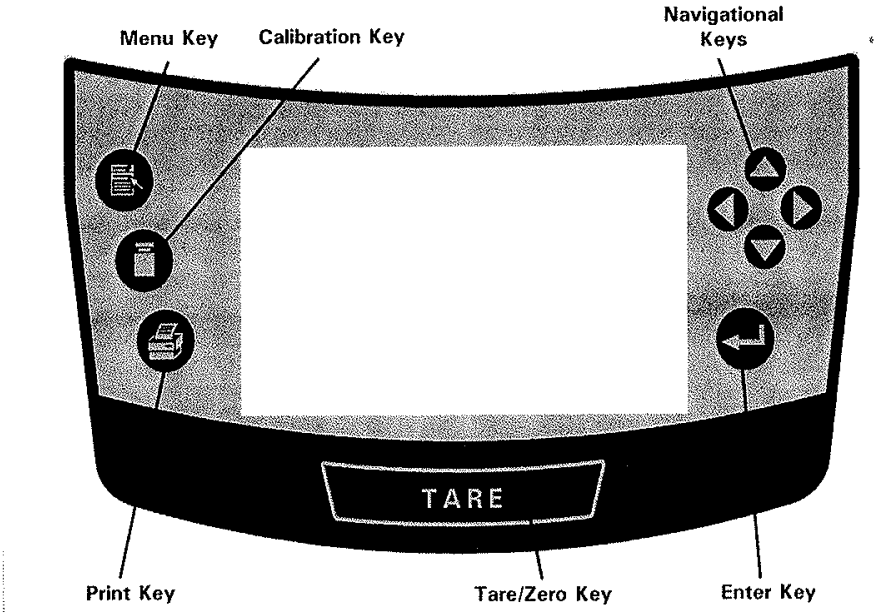


Figure 1: Denver Instrument PI 403
Denver Instrument PI 403 Manual

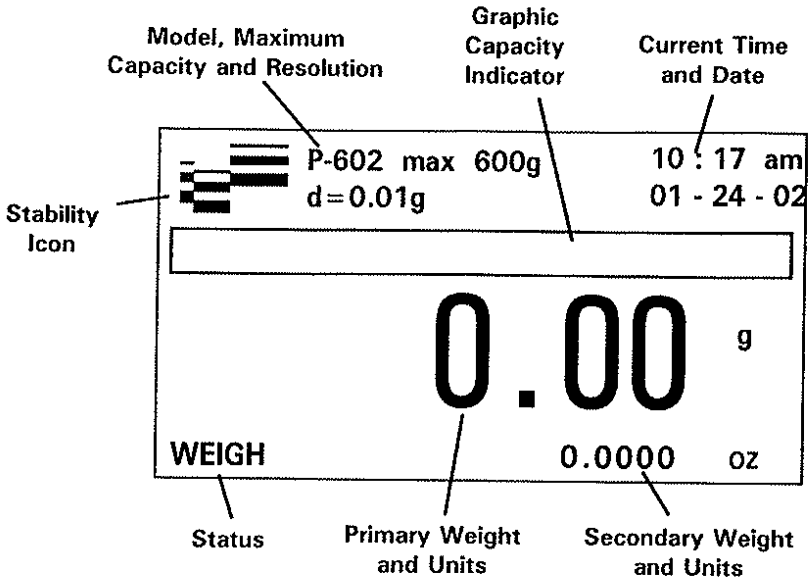


Figure 2: Denver Instrument PI 403 Display and Keyboard
Denver Instrument PI 403 Manual

SOP: Denver Instruments PI 403 Balance Calibration

10. History:

Revision Number	***Effective Date***	***Preparer***	***Description of Change***
0	09JAN14	Jason McMillan	Initial release

SOP: Denver Instrument PI 403 Balance Operation

Approvals:

Preparer: Jason McMillan Date: 10JAN14
Reviewer: Dr. Margaret Bryans Date: 11JAN14

1. **Purpose:**
   1.1. Operation of Denver Instrument PI 403 Balance.
2. **Scope:**
   2.1. Applies to the weight measurement of laboratory materials.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Denver Instrument PI 403 Balance Instruction Manual.
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. Appropriate weighing container(s) such as a weigh boat, weigh paper, beaker, flask, bottle, etc.
8. **Procedure:**
   8.1. **Simple Weighing**
      8.1.1. Place weighing container on the balance weighing pan. Press the "TARE" button to zero the weight display.
      8.1.2. **TARING** displayed on the screen indicates that the balance is waiting for the stable reading before taring.
      8.1.3. The balance will read 0.0000 grams (or selected units to designated resolution) after successful taring.
      8.1.4. When the reading is stable, the **Denver Instrument icon** appears in the top, left on the display. When unstable, a **U** appears.
      8.1.5. Remove the weighing container. Load weighing container with material to be weighed. Place loaded weighing container on the weighing pan. Weight for the **Denver Instrument icon** to appear in the upper left-hand corner.
      8.1.6. Record the weight and remove the loaded weighing container.

SOP: Denver Instrument PI 403 Balance Operation

9. Attachments:

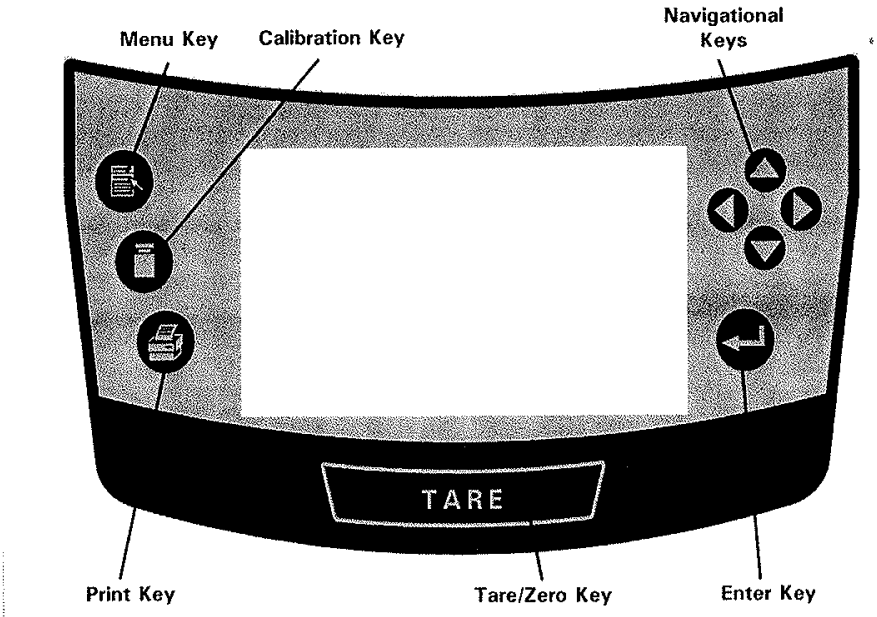


Figure 1: Denver Instrument PI 403
Denver Instrument PI 403 Manual

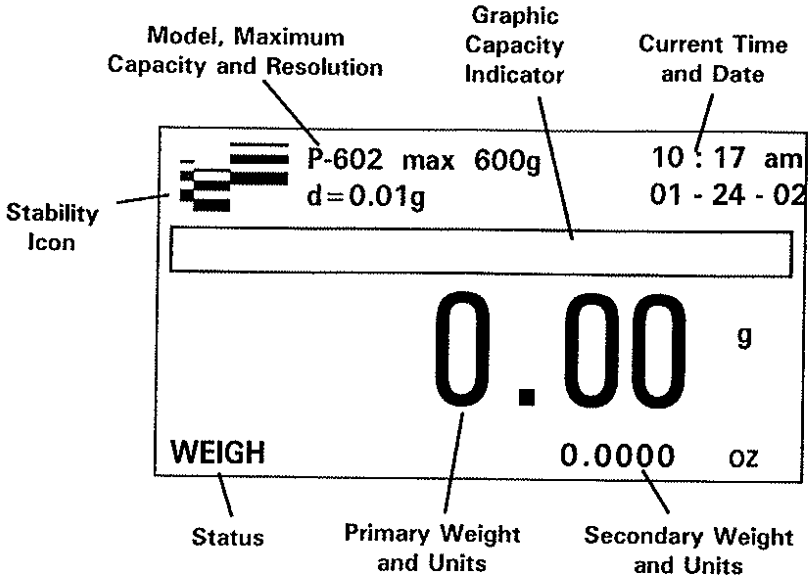


Figure 2: Denver Instrument PI 403 Display
Denver Instrument PI 403 Manual

SOP: Denver Instrument PI 403 Balance Operation

10. History:

Revision Number	***Effective Date***	***Preparer***	***Description of Change***
0	10JAN14	Jason McMillan	Initial release

SOP: Denver Instruments SI 4002 Balance Calibration

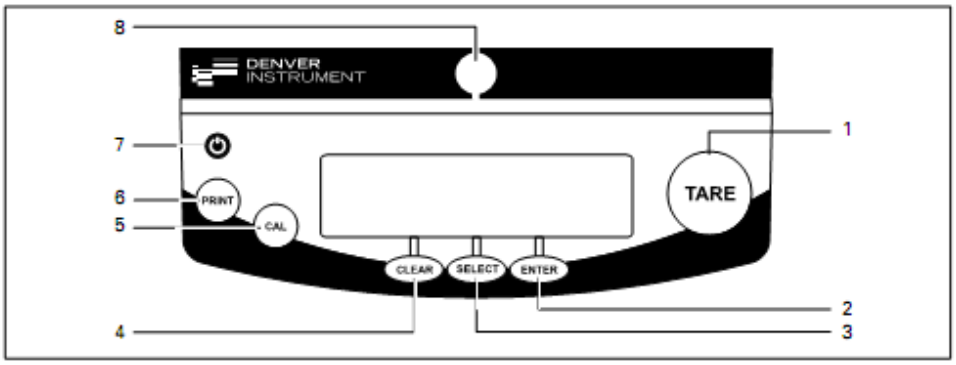
Approvals:

Preparer: Jason McMillan Date: 09JAN14
Reviewer: Dr. Margaret Bryans Date: 10JAN14

1. **Purpose:**
   1.1. Calibration of Denver Instruments SI 4002 Balance.
2. **Scope:**
   2.1. Applies to the calibration of Denver Instruments SI 4002 Balance.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Denver Instruments SI 4002 Balance Instruction Manual
   4.2. Denver Instruments SI 4002 Balance Operation SOP
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. Denver Instruments SI 4002 Balance
8. **Procedure:**
   8.1. **Internal Calibration**
      8.1.1. Press “ON/OFF” button to turn the balance on.
      8.1.2. Press the “TARE” button to tare the balance.
      8.1.3. Press the “CAL” button to start calibration. **CAL.INT.** will be displayed followed by **CAL.RUN.** while the built in weight is applied automatically.
      8.1.4. When calibration is finished the display will read **CAL.END** and the balance is ready to use.

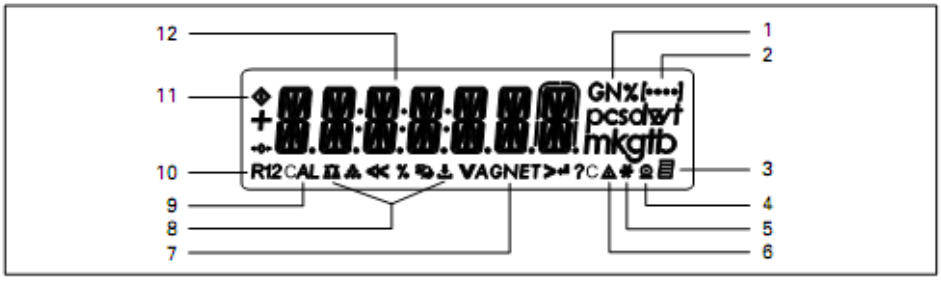
SOP: Denver Instruments SI 4002 Balance Calibration

9. Attachments:



Pos.	Designation
1	Taring
2	Start an application program
3	Select an application program | Open the operating menu
4	Clear This key is generally used to cancel functions: – Quit application program – Cancel calibration/adjustment routine | Exit the operating menu
5	Start calibration/adjustment routine
6	Data output: Press this key to send readout values to the built-in data interface.
7	On/off
8	Level indicator

Figure 1: Denver Instrument SI 4002
Denver Instrument SI 4002 Manual



Pos.	Designation
1	Weight unit
2	Menu level indicator
3	Symbol: "GLP printing mode active"
4	Symbol: "Printing mode active"
5	Symbol: "Application program active"
6	Calculated-value indicator (i.e., not a weight value)
7	Symbol: Gross or net value
8	Symbols for active application (⚖, ⁂, %, ⚖, ⚓, A, C)
9	Symbol: Calibration/adjustment function
10	Symbols for zero range
11	Busy symbol: command is being processed (for example, "Wait for stability icon); after you turn on the power, ◈ will be displayed until you press a key
12	Weight value displayed in selected weight unit
Symbols:	
<<	Save settings and exit the operating menu
<	One menu level higher
V	Scroll through menu items
>	Next item on current menu level
↵	Select a parameter setting

Figure 2: Denver Instrument SI 4002 Display
Denver Instrument SI 4002 Manual

10. History:

Revision Number	*Effective Date*	*Preparer*	*Description of Change*
0	09JAN14	Jason McMillan	Initial release

SOP: Denver Instrument SI 4002 Balance Operation

Approvals:

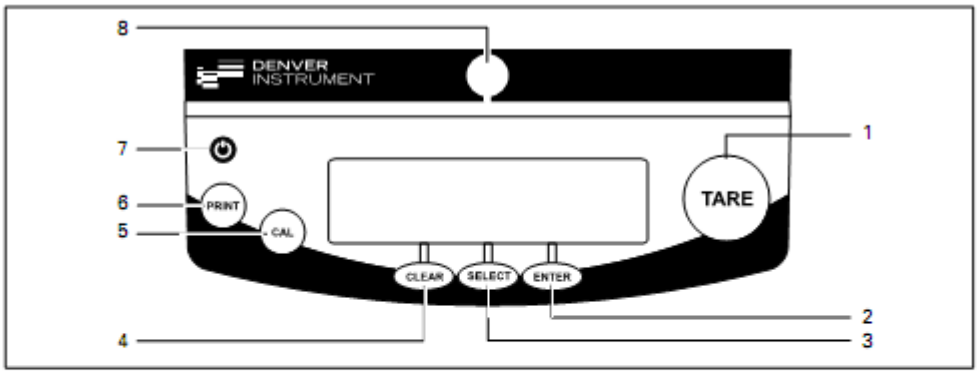
Preparer: Jason McMillan Date: 09JAN14
Reviewer: Dr. Maggie Bryans Date: 10JAN14

1. **Purpose:**
   1.1. Operation of Denver Instrument SI 4002 Balance.
2. **Scope:**
   2.1. Applies to the weight measurement of laboratory materials.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor /lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Denver Instrument SI 4002 Balance Instruction Manual.
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. Appropriate weighing container(s) such as a weigh boat, weigh paper, beaker, flask, bottle, etc.
8. **Procedure:**
   8.1. **Simple Weighing**
      8.1.1. Turn the scale on using the "ON/OFF" button.
      8.1.2. Place weighing container on the balance weighing pan. Press the "TARE" button to zero the weight display.
      8.1.3. Remove the weighing container. Load weighing container with material to be weighed. Place loaded weighing container on the weighing pan. Read off the weight indicated on the display when **g** appears on the right side of the weight.
      8.1.4. Remove the loaded weighing container.
      8.1.5. To turn balance off, press and hold the "ON/OFF" button.
      8.1.6. Clean off the top of the balance using the balance cleaning brush being sure to apply minimal pressure.

9. **Troubleshooting**
   9.1. See Table 1

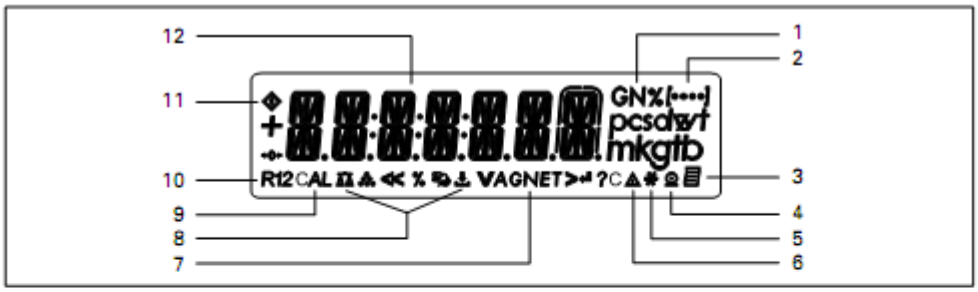
SOP: Denver Instrument SI 4002 Balance Operation

10. Attachments:



Pos.	Designation
1	Taring
2	Start an application program
3	Select an application program | Open the operating menu
4	Clear This key is generally used to cancel functions: – Quit application program – Cancel calibration/adjustment routine | Exit the operating menu
5	Start calibration/adjustment routine
6	Data output: Press this key to send readout values to the built-in data interface.
7	On/off
8	Level indicator

Figure 1: Denver Instrument SI 4002
Denver Instrument SI 4002 Manual



Pos.	Designation
1	Weight unit
2	Menu level indicator
3	Symbol: "GLP printing mode active"
4	Symbol: "Printing mode active"
5	Symbol: "Application program active"
6	Calculated-value indicator (i.e., not a weight value)
7	Symbol: Gross or net value
8	Symbols for active application (ΔT, ⁂, %, ⚖, ⊥, A, C)
9	Symbol: Calibration/adjustment function
10	Symbols for zero range
11	Busy symbol: command is being processed (for example, "Wait for stability icon"); after you turn on the power, ⏻ will be displayed until you press a key
12	Weight value displayed in selected weight unit

Symbols:

<<	Save settings and exit the operating menu
<	One menu level higher
V	Scroll through menu items
>	Next item on current menu level
↵	Select a parameter setting

SOP: Denver Instrument SI 4002 Balance Operation

Figure 2: Denver Instrument SI 4002 Display
Denver Instrument SI 4002 Manual

Troubleshooting Guide

Error codes are shown on the main display for approx. 2 seconds. The program then returns automatically to the previous mode.

Display	Cause	Solution
No segments appear on the display	No AC power is available	Check the AC power supply
	The power supply is not plugged in	Plug in the power supply
HIGH	The load exceeds the balance capacity	Unload the balance
LOW or ERR 54	Something is touching the weighing pan	Move the object that is touching the weighing pan
ERR 54, typical	Weighing system defect	Contact Denver Service Center
APPERR.	Cannot store data: Load on weighing pan too light or no sample on pan while application is active	Increase load
DISERR.	Data output not compatible with output format	Change the configuration in the operating menu
PRTERR.	Interface port for printer output is blocked	Reset the menu factory settings, or Contact your local Denver Service Center
ERR 02	Calibration parameter not met; e.g.: – balance not tared – load on weighing pan	Calibrate only when zero is displayed – Press (TARE) to tare the balance – Unload the balance
ERR 10	The (TARE) key is blocked when there is data in the second tare memory (net-total); only 1 tare function can be used at a time	Press (CLEAR) to clear the tare memory and release the tare key
ERR 11	Tare memory not allowed	Press (TARE)
The weight readout changes constantly	Unstable ambient conditions (excessive vibration or draft) at the place of installation	Set up the balance in another area
	A foreign object is caught between weighing pan and balance housing	Remove the foreign object
The weight readout is obviously wrong	The balance was not calibrated/adjusted	Calibrate/adjust the balance
	Balance not tared before weighing	Tare or zero the balance before weighing

Table 1: Troubleshooting Guide
Denver Instrument SI 4002 Manual

SOP: Denver Instrument SI 4002 Balance Operation

11. History:

Revision Number	***Effective Date***	***Preparer***	***Description of Change***
0	09JAN14	Jason McMillan	Initial release

SOP: Fisher Accu 124 Balance Calibration

Approvals:
Preparer: Jason McMillan Date: 14JAN14
Reviewer: Dr. Margaret Bryans Date: 15JAN14

1. **Purpose:**
   1.1. Operation of Fisher Accu 124 Balance.
2. **Scope:**
   2.1. Applies to the weight measurement of laboratory materials.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Fisher Accu 124 Balance Instruction Manual.
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. Fisher Accu 124 Balance
   7.2. 100g ASTM Class 1 Standard Mass
8. **Procedure:**
   8.1. **Internal Calibration**
      8.1.1. Press the "TARE" button to zero the weight display **TARING** will be displayed.
      8.1.2. Press the "CALIBRATION" button. **CAL** will be displayed during calibration.
      8.1.3. When calibration is complete the unit will return to the measurement mode.
   8.2. **External Calibration**
      8.2.1. Press the "TARE" button to zero the weight display.
      8.2.2. Gently place the 100g ASTM Class 1 mass standard on the balance. **Be sure to use utensils, do not use your hand**.
      8.2.3. Press the "CALIBRATION" button.
      8.2.4. The display will read **CAL**.
      8.2.5. When calibration is complete the display will read **100g** and the unit will return to measurement mode.

SOP: Fisher Accu 124 Balance Calibration

9. Attachments:

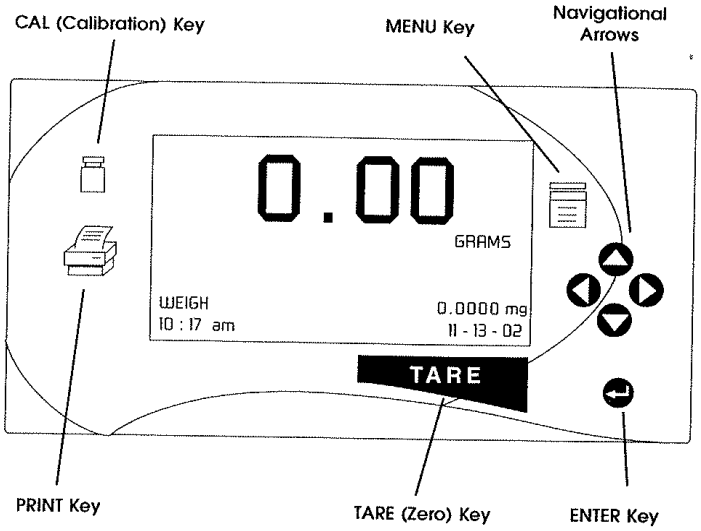


Figure 1: Fisher Accu 124
Fisher Accu 124 Manual

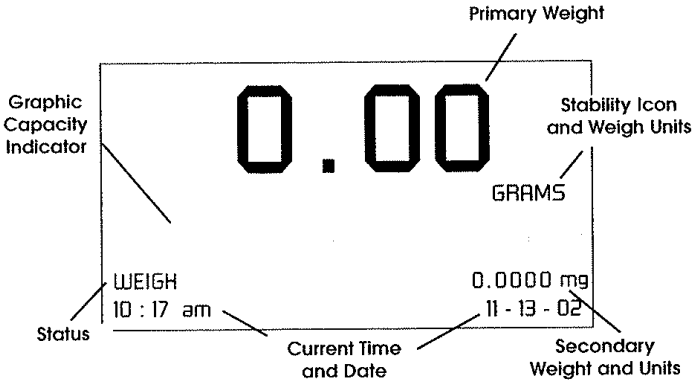


Figure 2: Fisher Accue 124 Display
Fisher Accu 124 Manual

10. History:

Revision Number	***Effective Date***	***Preparer***	***Description of Change***
0	14JAN14	Jason McMillan	Initial release

SOP: Fisher Accu 124 Balance Operation

Approvals:

Preparer: Jason McMillan Date: 14JAN14
Reviewer: Dr. Maggie Bryans Date: 15JAN14

1. **Purpose:**
   1.1. Operation of Fisher Accu 124 Balance.
2. **Scope:**
   2.1. Applies to the weight measurement of laboratory materials.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Fisher Accu 124 Balance Instruction Manual.
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. Appropriate weighing container(s) such as weigh boat, weigh paper, beaker, flask, bottle, etc.
8. **Procedure:**
   8.1. **Simple Weighing**
      8.1.1. Place weighing container on the balance weighing pan. Press the "TARE" button to zero the weight display **TARING** will be displayed and then the display will read **0.0000g** (or selected units to the designated resolution). When the reading is stable the weigh units will appear under the weight reading on the display.
      8.1.2. Remove the weighing container. Load weighing container with material to be weighed. Place loaded weighing container on the weighing pan. When the reading is stable the weigh units will appear under the weight reading on the display. Record the weight indicated on the display.
      8.1.3. Remove the loaded weighing container.

SOP: Fisher Accu 124 Balance Operation

9. Attachments:

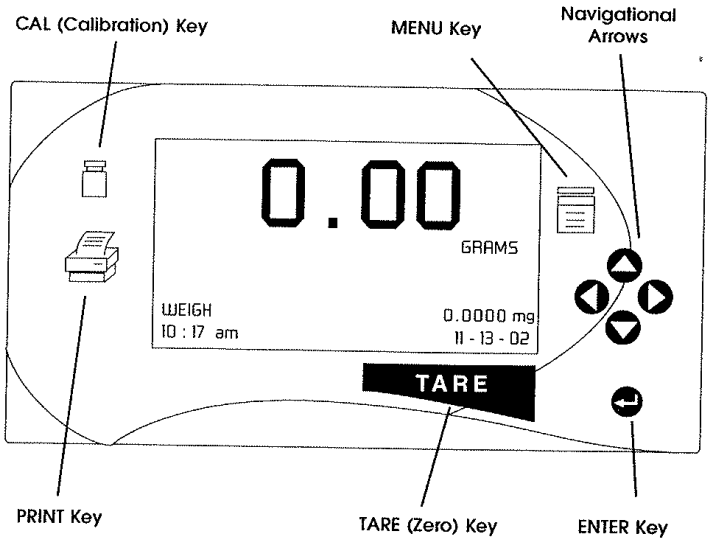


Figure 1: Fisher Accu 124
Fisher Accu 124 Manual

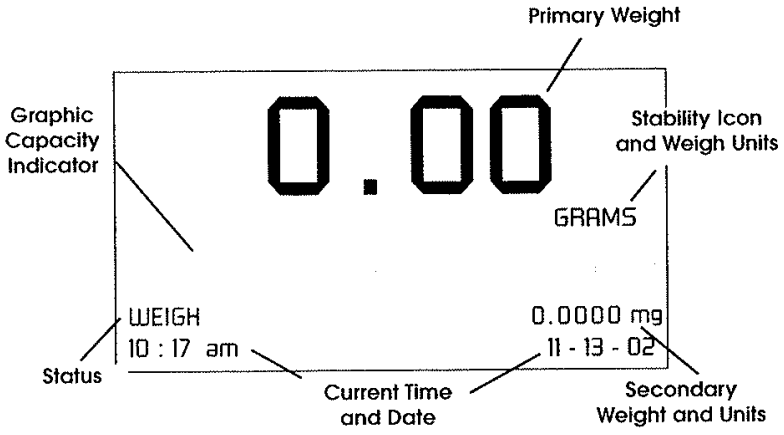


Figure 2: Fisher Accue 124 Display
Fisher Accu 124 Manual

10. History:

Revision Number	*Effective Date*	*Preparer*	*Description of Change*
0	14JAN14	Jason McMillan	Initial release

SOP: Fisher Accu 124D Balance Calibration

Approvals:

Preparer: Jason McMillan — Date: 14JAN14
Reviewer: Dr. Maggie Bryans — Date: 15JAN14

1. **Purpose:**
   1.1. Operation of Fisher Accu 124D Balance.
2. **Scope:**
   2.1. Applies to the weight measurement of laboratory materials.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor /lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Fisher Accu 124D Balance Instruction Manual.
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. Fisher Accu 124D Balance
8. **Procedure:**
   8.1. Internal Calibration
   8.1.1. Press the ON/OFF button if the balance is not on.
   8.1.2. Press the "TARE" button to zero the weight display.
   8.1.3. Press the "CAL/CF" button. **C CAL** will be displayed during calibration.
   8.1.4. When calibration is complete the unit will return to the measurement mode.

SOP: Fisher Accu 124D Balance Calibration

9. Attachments:

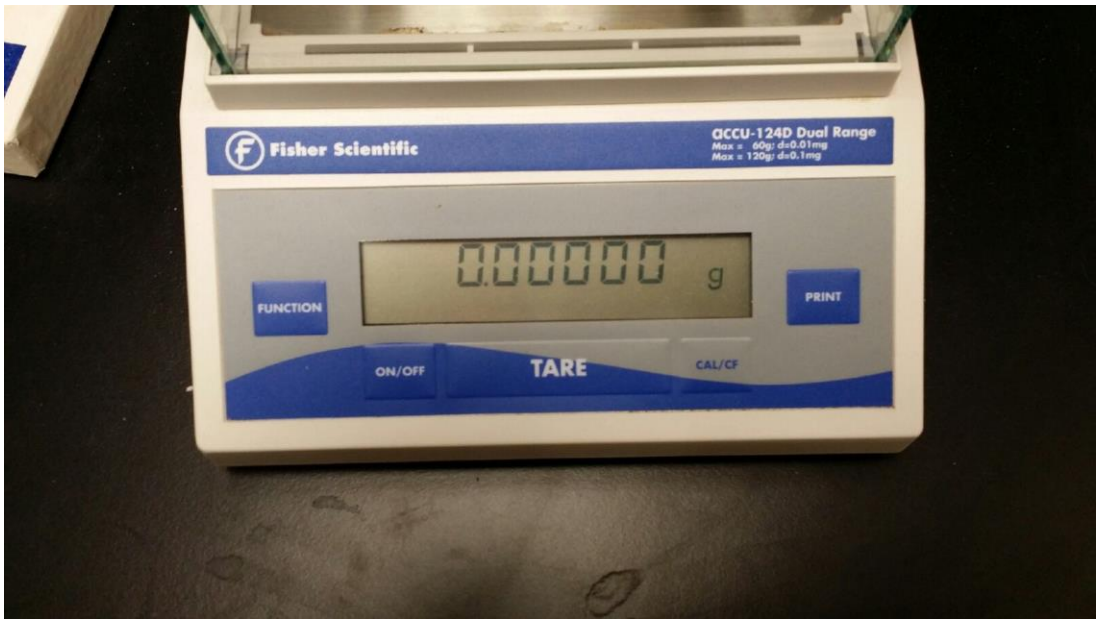


Figure 1: Fisher Accu 124D

10. History:

Revision Number	*Effective Date*	*Preparer*	*Description of Change*
0	14JAN14	Jason McMillan	Initial release

SOP: Fisher Accu 124D Balance Operation

Approvals:

Preparer: Jason McMillan — Date: 14JAN14
Reviewer: Dr. Margaret Bryans — Date: 15JAN14

1. **Purpose:**
   1.1. Operation of Fisher Accu 124D Balance.
2. **Scope:**
   2.1. Applies to the weight measurement of laboratory materials.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Fisher Accu 124D Balance Instruction Manual.
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. Appropriate weighing container(s) such as a weigh boat, weigh paper, beaker, flask, bottle, etc.
   7.2. Fisher Accu 124D Balance
8. **Procedure:**
   8.1. Simple Weighing
   8.1.1. Press the "ON/OFF" button if balance is not on.
   8.1.2. Place weighing container on the balance weighing pan. Press the "TARE" button to zero the weight display. The display will read **0.0000g** (or selected units to the designated resolution). When the reading is stable the weigh units will appear under the weight reading on the display.
   8.1.3. Remove the weighing container. Load weighing container with material to be weighed. Place loaded weighing container on the weighing pan. When the reading is stable the weigh units will appear under the weight reading on the display. Record the weight indicated on the display.
   8.1.4. Remove the loaded weighing container.

SOP: Fisher Accu 124D Balance Operation

9. Attachments:

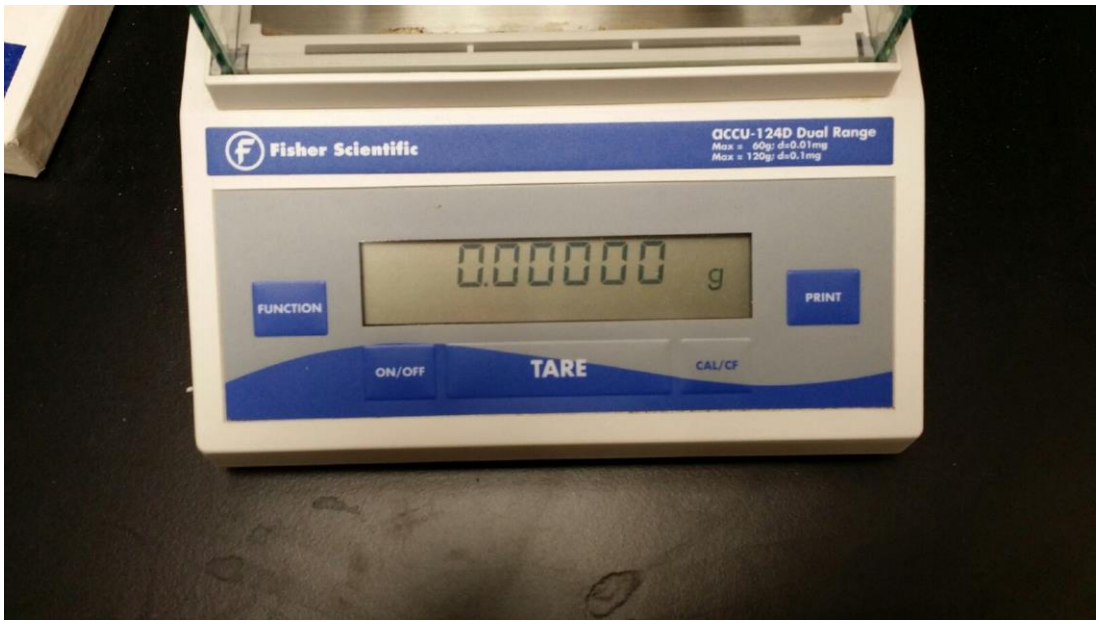


Figure 1: Fisher Accu 124D

10. History:

Revision Number	*Effective Date*	*Preparer*	*Description of Change*
0	14JAN14	Jason McMillan	Initial release

FORM: Balance Calibration

Balance Information

Name and Description: _____

Model: _____

Serial Number:_____

Calibration Information

Date of Calibration: _____ **Technician:_____**

Standard Mass Weights Used:_____

Comments:_____

Passed Calibration:	**Failed Calibration:**
Pass: □Yes □ No Calibration Sticker: □Yes □ No □ Not Applicable Next Calibration Due Date:_____	Reason for Failure:_____ Date Out of Service:_____ Initials:_____

SOP: Eppendorf Research Plus Pipette Operation and Maintenance

Approvals:

Preparer: Jason McMillan Date: 08JAN14
Reviewer: Dr. Maggie Bryans Date: 10JAN14

1. **Purpose:**
   Operation of Eppendorf Research Plus through to the P-1000.
2. **Scope:**
   Applies to the operation, cleaning, and trouble shooting of the Eppendorf Research Plus, designed to dispense precise volumes of liquid safely.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Eppendorf Research Plus Operating Manual
   4.1. Tuttnauer 3850 ELV Autoclave SOP
5. **Definitions:** N/A
6. **Precautions:**
   6.1. Volatile solutions: you should saturate the air-cushion of your pipette by aspirating and dispensing the solvent repeatedly before aspirating the sample.
   6.2. Acids or other corrosive liquids that emit vapors; can damage pipette to avoid this remove the tip holder and rinse the piston and O-ring and seal with distilled water.
   6.3. Temperature extremes can damage the Eppendorf Research Plus. Do not pipette liquids having temperatures of above 70^0C or below 4^0C.
7. **Materials:**
   7.1. Eppendorf Research Plus
   7.2. pipette tips
   7.3. beaker
   7.4. weigh boats
   7.5. MilliQ Water
   7.6. lab towels
   7.7. 70% isopropyl alcohol (IPA)
   7.8. autoclave
8. **Procedure:**
   8.1. **Operation**
      8.1.1. The volume of liquid to be aspirated is set using the “Volume Display” which is read top (most significant digit) to bottom (least significant digit).
      8.1.2. Turn the “Volume Adjustment Ring” to select the desired volume. To obtain the maximum accuracy when setting the volume, set the volume 1/3 of a turn above the desired volume and then turn down to the desired volume.
      8.1.3. Double check that the set volume is correct while holding the “Volume Display” at eye level.

SOP: Eppendorf Research Plus Pipette Operation and Maintenance

8.1.4. Fit a tip into the tip holder, by using a slight twisting motion when pressing the Eppendorf Research Plus "Spring Loaded Tip Cone" into a pipette tip to ensure a firm and airtight seal. The pipette tip is securely attached to the "Spring Loaded Tip Cone" when it responds with spring loaded action.

8.1.5. Pre-rinse the tip by aspirating the first volume of liquid and then dispensing it back into the sample container or a waste container.

8.2. **Aspirating Liquid**

8.2.1. Press down the "Control Button" to the first stop (measuring stroke).

8.2.2. Immerse the pipette tip vertically approximately 4mm into the liquid.

8.2.3. To aspirate the liquid, allow the "Control Button" to slide back slowly. Maintain the immersion depth to prevent accidental air aspiration.

8.2.4. When pipetting large volumes wait approximately 3 seconds before removing the pipette tip from the liquid. To ensure maximum precision and accuracy the manufacturers recommends wetting each new tip initially by aspirating and dispensing the liquid 1-3 times and then commence pipetting.

8.2.5. Remove the tip slowly from the liquid making sure to slowly wipe the tip against the tube wall to ensure that no outer wetting remains on the tip.

8.3. **Dispensing Liquid**

8.3.1. Place the tip on the tube wall at an angle and press down the "Control Button" to the first stop (measuring stroke) and wait until the flow of liquid stops. Then Press the "Control Button" until it reaches the second stop (blow out) to empty the tip completely.

8.3.2. Continue holding down the "Control Button" and wipe the tip against the tube inner wall and remove the tip from the tube. Let the "Control Button" slide back slowly once outside of the tube.

8.4. **Tip removal**

8.4.1. Tip may now be ejected by pressing firmly on the "Ejector" button into a waste container.

8.4.2. Tip changes are required only if aspirating a different liquid, sample or reagent or volume. Tips should also be changed if aseptic technique is compromised (e.g. if the tip touches the outside of a container).

8.4.3. When you are finished pipetting, re-set the volume of the Eppendorf Research Plus to the maximum volume for proper storage.

8.5. **Leak testing P20 – P200**

8.5.1. Fit a tip onto the Eppendorf Research Plus pipette.

8.5.2. Set to the maximum volume given in the specification.

8.5.3. For volumes <20µl pre-wet the tip several times.

8.5.4. Hold the Eppendorf Research Plus in a vertical position with a full tip for approximately 30 seconds. Do not touch the pipette tip.

8.5.5. Observe the meniscus of the liquid on the tip opening. If there is a leak in the pipette, a droplet will form on the tip opening.

8.5.6. Verify that the tip is on tightly, and repeat the test.

8.5.7. If a droplet appears at the end of the tip there is a leak and the Eppendorf Research Plus needs repair.

SOP: Eppendorf Research Plus Pipette Operation and Maintenance

8.6. **Clean the pipette**

Note: Most pipettes are designed so that the parts that normally come into contact with liquid contaminants can easily be cleaned and decontaminated.

8.6.1. Wipe entire pipette with a lab towel dampened with a mild detergent solution.
8.6.2. Wipe entire pipette with a lab towel dampened with MilliQ water.
8.6.3. Remove the ejector sleeve by holding down the ejection button and pulling on the ejector sleeve (Figure 2: Step 1).
8.6.4. Slide up the ring on the lower part with the label "PUSH TO RELEASE" approximately 5mm until the lower part is released (Figure 2: Step 2 & 3).
8.6.5. The lower part is then removed from the upper part (Figure 2: Step 4).
8.6.6. Wipe the ejector sleeve and lower part with a lab towel dampened with a mild soap solution or 70% IPA.
8.6.7. Wipe the ejector sleeve and lower part with a lab towel dampened with MilliQ water.
8.6.8. Refit the lower part into the upper part until it engages audibly.
8.6.9. Refit the ejector sleeve and allow the pipette to dry.
8.6.10. Dispose of lab towels in bio-hazardous waste receptacle.

8.7. **Chemical decontamination**

8.7.1. Spray a lab towel with 70% IPA to dampen the lab towel.
8.7.2. Wipe upper part of body with dampened lab towel.
8.7.3. Wipe ejector sleeve with dampened lab towel.
8.7.4. Wipe entire pipette with a lab towel dampened with MilliQ water.
8.7.5. Leave pipette to dry or wipe pipette dry with lab towel.
8.7.6. Dispose lab towels in bio-hazardous waste receptacle.

8.8. **Autoclaving**

8.8.1. Place the whole Eppendorf research Plus unit into the autoclave
8.8.2. Run the autoclave on the Unwrapped Delicate Instruments per Tuttnauer 3850 ELV Autoclave SOP
8.8.3. Remove the Eppendorf Research Plus unit and allow it to dry completely and cool Down.

8.9. **Trouble shooting**

8.9.1. See the trouble shooting table (Table 1).

SOP: Eppendorf Research Plus Pipette Operation and Maintenance

9. Attachments:

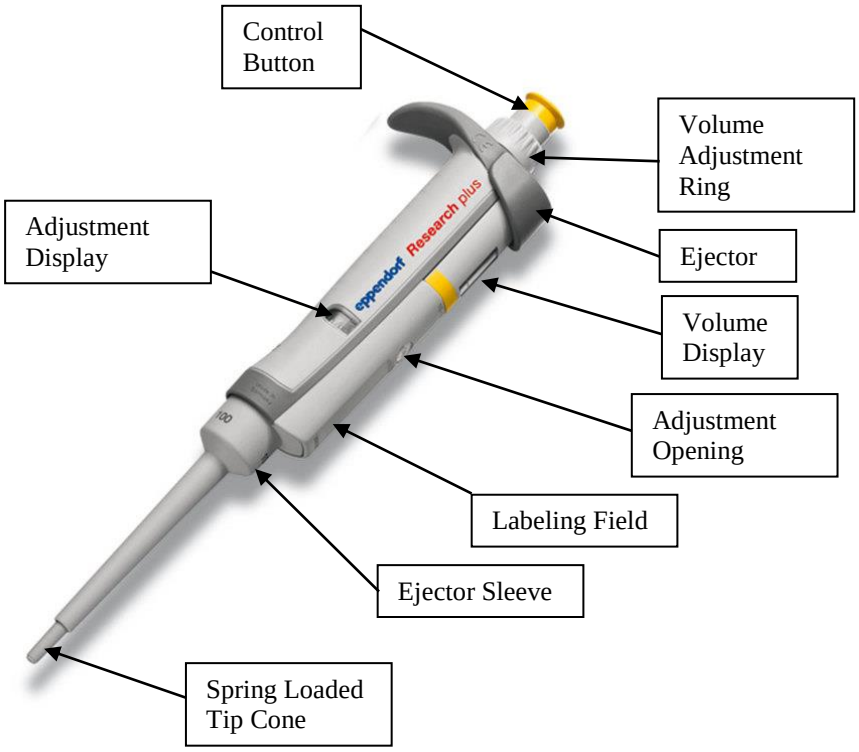


Figure 1: Eppendorf Research Plus

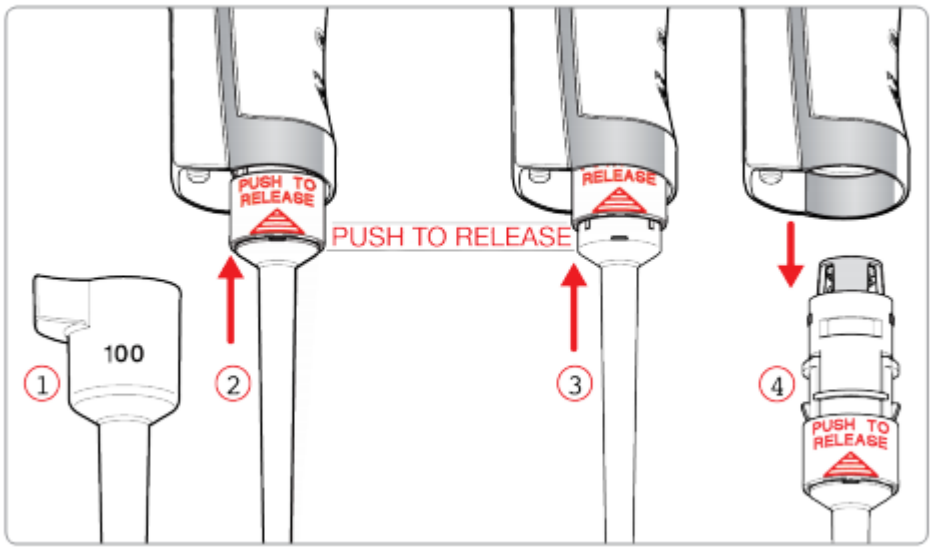


Figure 2: Removing the Lower Part
Eppendorf Research Plus Manual

SOP: Eppendorf Research Plus Pipette Operation and Maintenance

Symptom	Possible cause	Solution
Liquid is dripping from the tip and/or the dispensed volume is incorrect.	• The tip is loose or the pipette tip is poorly fitted.	➢ Press the tip on firmly.
	• Liquid with high vapor pressure and/or different density.	➢ Wet the tip several times and adjust the pipette for the liquid used.
	• Pipetted too quickly.	➢ Move the control button slowly.
	• The tip is withdrawn from the liquid too quickly.	➢ Slowly remove the tip with a time delay (approximately 3 seconds) from the liquid.
	• Liquid aspirated with blow-out and dispensed with blow-out.	➢ Repeat dispensing correctly.
	• The piston is soiled or damaged.	➢ Clean the piston, relubricate slightly and/or replace.
	• The tip cone is damaged.	➢ Replace the lower part or channel.
	• The O-rings of the tip cones are damaged.	➢ Replace the O-rings (only 100 µl, 300 µl multi-channel).
The control button jams and does not move smoothly.	• The piston is soiled. • The seal is soiled. • The pipette is blocked.	➢ Clean the lower part. ➢ 5ml and 10ml sizes; replace the protection filter.
The adjustment seal has been removed; the adjustment display has been changed.	• The pipette has been adjusted for another liquid.	➢ Adjust the pipette for the liquid used.
No spring-loading action of the tip cone when taking up pipette tips.	• Spring-loading action is blocked by a locking ring.	➢ Remove the locking ring again.
	➢ The use of a 5ml or 10ml pipette.	➢ No remedy. The tip cone does not respond with spring-loaded action in combination with these sizes.

Table 1: Trouble Shooting
Eppendorf Research Plus Manual

10. History:

Revision Number	***Effective Date***	***Preparer***	***Description of Change***
0	08JAN14	Jason McMillan	Initial release

SOP: Eppendorf Research Plus Performance Verification SOP

Approvals:

Preparer: Jason McMillan — Date: 08JAN14
Reviewer: Dr. Maggie Bryans — Date: 10JAN14

1. **Purpose:** To verify the calibration of a single channel pipette.
2. **Scope:** Covers the cleaning, decontamination and verification of a single channel pipette.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. balance operation SOP
   4.2. balance calibration SOP
   4.3. Tuttnauer 3850 ELV Autoclave SOP
   4.4. Eppendorf Research Plus Operation and Maintenance SOP
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. balance
   7.2. weigh boats
   7.3. MilliQ water
   7.4. small beaker for holding MilliQ water
   7.5. verification labels
   7.6. verification form
   7.7. verification Pass/Fail form
   7.8. pipette tips
   7.9. Eppendorf Research Plus (P20, P200, and P1000)
   7.10. 70% isopropyl alcohol (IPA)
   7.11. lab towels
   7.12. tweezers
   7.13. thermometer
   7.14. calculator
   7.15. barometer
8. **Procedure:**
   8.1. **Clean the pipette** (See Figure 2.)
   Note: Most pipettes are designed so that the parts that normally come into contact with liquid contaminants can easily be cleaned and decontaminated.
      8.1.1. Wipe entire pipette with a lab towel dampened with a mild detergent solution.
      8.1.2. Wipe entire pipette with a lab towel dampened with distilled water.
      8.1.3. Remove the ejector sleeve by holding down the ejection button and pulling on the ejector sleeve (Figure 2: Step 1).
      8.1.4. Slide up the ring on the lower part with the label “PUSH TO RELEASE”

SOP: Eppendorf Research Plus Performance Verification SOP

approximately 5mm until the lower part is released (Figure 2: Step 2 & 3).

8.1.5. The lower part is then removed from the upper part (Figure 2: Step 4).

8.1.6. Wipe the ejector sleeve and lower part with a lab towel dampened with a mild soap solution or 70% IPA.

8.1.7. Wipe the ejector sleeve and lower part with a lab towel dampened with MilliQ water.

8.1.8. Refit the lower part into the upper part until it engages audibly.

8.1.9. Refit the ejector sleeve and allow the pipette to dry.

8.1.10. Dispose of lab towels in bio-hazardous waste receptacle.

8.2. **Chemical decontamination**

8.2.1. Spray a lab towel with 70% IPA to dampen the lab towel.

8.2.2. Wipe upper part of body with dampened lab towel.

8.2.3. Wipe ejector sleeve with dampened lab towel.

8.2.4. Wipe entire pipette with a lab towel dampened with MilliQ water.

8.2.5. Leave pipette to dry or wipe pipette dry with lab towel.

8.2.6. Dispose lab towels in bio-hazardous waste receptacle.

8.3. Autoclaving

8.3.1. Place the whole Eppendorf research Plus unit into the autoclave

8.3.2. Run the autoclave on the Unwrapped Delicate Instruments per Tuttnauer 3850 ELV Autoclave SOP

8.3.3. Remove the Eppendorf research Plus unit and allow it to dry completely and cool down.

8.4. **Verification of Calibration**

Note: To test the accuracy of the pipette you will pipette a set volume 10 times and then weigh the total pipetted volume. 1mL of MilliQ water should weigh 1g and 1µL should weigh 1mg. Calculate your % Error using the equation below:

$$\frac{\text{Expected Mass - Actual Mass}}{\text{Expected Mass}} \times 100 = \%\ \text{Error}$$

If the % Error is ≤ 2% the pipette passes verification, if the % Error is > than 2% the pipette fails. We will verify the pipette once at the maximum volume for the pipette, once at the ½ maximum volume, and once at the minimum volume. Altogether you will pipette 30 volumes and weigh 3 times for each pipette.

8.4.1. Record the necessary information on the Verification form. Enter information in the empty box to the right of the box specifying the information.

8.4.2. Verify that the calibration label of the balance is within the dated calibration time period.

8.4.3. Fill a small beaker with MilliQ water.

8.4.4. Place the weigh boat on the balance.

8.4.5. Tare the balance and verify that 0.00 is being displayed.

8.4.6. Verify that the pipette is set to the maximum volume (e.g. the maximum volume for a P-20 pipette is 20µL.).

SOP: Eppendorf Research Plus Performance Verification SOP

8.4.7. On the Pipette Verification Form, beside Selected Volume, enter the volume you will be pipetting and the value of that volume times 10 (e.g. for a 20µL pipette you will record 20µL for the selected volume and 200µL for the selected volume times 10.).
8.4.8. Calculate the expected mass by converting the selected volume times 10 using the following conversions: 1µL = 1mg and 1mL = 1g. Use the selected volume times 10 as the volume (e.g. for a 20µL pipette, 200µL multiplied by 1mg/µL = 200mg). Record the expected mass in the box beside Expected Mass.
8.4.9. Verify that the pipette is set to the maximum volume recommended by the manufacturer for the pipette.
8.4.10. Place pipette tip securely on the pipette.
8.4.11. Aspirate MilliQ water into pipette tip from the beaker and dispense it into weigh boat.
8.4.12. Repeat the above step 9 times. Each time you dispense the selected volume mark the Verification form in the numbered box beside Dispense Repetitions.
8.4.13. Record the final mass on the Verification form next to Recorded Mass.
8.4.14. Tare the balance and verify that 0.00 is being displayed.
8.4.15. Set the volume of the pipette to half capacity (e.g. For a P-20 pipette, set it to 10µL.) and verify the volume.
8.4.16. Repeat steps 8.4.9. through 8.4.16 with the pipette set to the half-capacity volume.
8.4.17. Tare the balance and verify that 0.00 is being displayed.
8.4.18. Set the volume of the pipette to the minimum capacity recommended by the manufacturer (e.g. For a P-20 pipette, set it to 2µL.)
8.4.19. Repeat steps 8.4.9. through 8.4.16 with the pipette set to the minimum-capacity volume.
8.4.20. Calculate the % Error (as directed in the note at the beginning of section 8.4) for each test (maximum, half-capacity, and minimum volumes) and record the results on the verification form.
8.4.21. Verify that all fields of the verification form have been filled out and fill out the Pipette Verification Pass/Fail form according to the results of the tests.

9. Attachments:

SOP: Eppendorf Research Plus Performance Verification SOP

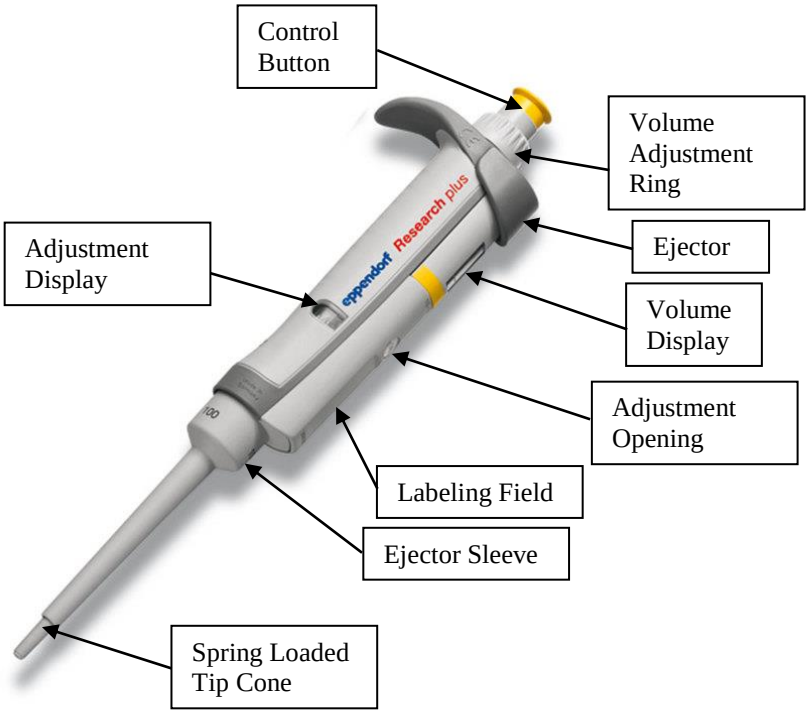


Figure 1: Eppendorf Research Plus

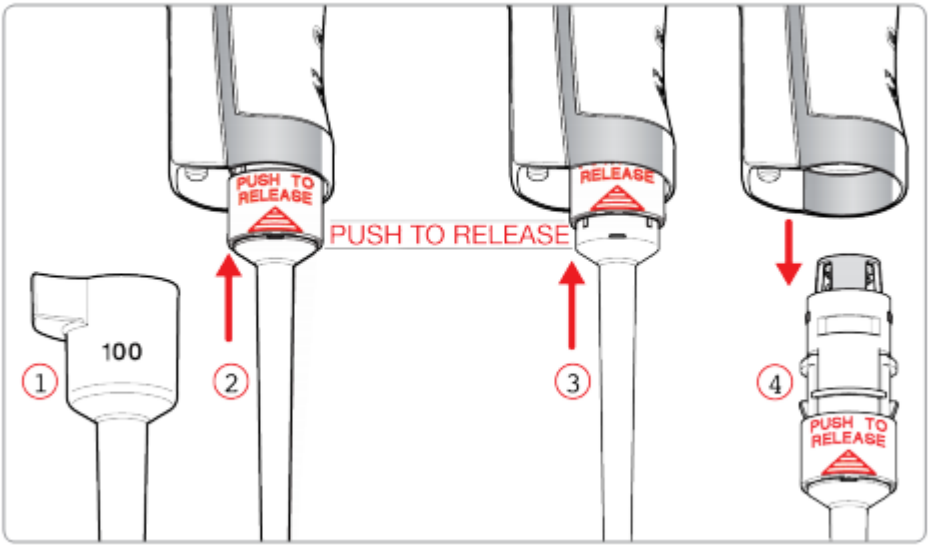


Figure 2: Removing the Lower Part
Eppendorf Research Plus Manual

SOP: Eppendorf Research Plus Performance Verification SOP

10. History

Revision Number	***Effective Date***	***Preparer***	***Description of Change***
0	08JAN14	Jason McMillan	Initial release
1	10JAN14	Jason McMillan	Added pipette verification form

SOP: Eppendorf Research Plus Performance Verification SOP

Pipette Information

Name and Description:_____

Model:_____

Serial Number:_____

Verification		**Pipette**	
Technician		Volume Range	
Date		Number of Channels	

Test Conditions			
Balance Serial #		Balance Model	
Sensitivity		Balance Calibration Date	
Correction Factor		Balance Calibration Technician	
Air Temperature			
Barometric Temperature			
Relative Humidity			

Tests												
Test 1 (Max. volume)												
Selected Volume											Expected Mass	
Selected Volume X 10											Recorded Mass	
Dispense Repetitions	1	2	3	4	5	6	7	8	9	10		
Test 2 (Half cap. volume)												
Selected Volume											Expected Mass	
Selected Volume X 10											Recorded Mass	
Dispense Repetitions	1	2	3	4	5	6	7	8	9	10		
Test 3 (Min. volume)												
Selected Volume											Expected Mass	
Selected Volume X 10											Recorded Mass	
Dispense Repetitions	1	2	3	4	5	6	7	8	9	10		

Test results	
% Error Test 1	
% Error Test 2	
% Error Test 3	
Pass or Fail	

FORM: Pipette Performance Verification Pass/Fail

Pipette Information

Name and Description: _____

Model: _____

Serial Number:_____

Verification Comments:

_____

_____

_____

Technician:_____

Date:_____

Passed Verification:	**Failed Verification:**
Date of Verification_____ Technician_____ Verification Sticker: □Yes □ No □ Not Applicable Next Verification Due Date_____	Reason for Failure_____ _____ _____ Date Out of Service_____ Technician_____

Passed Verification:
Date of **Verification**_______________
Technician_______________________
Calibration Sticker: ☐ Yes
☐ No
☐ Not Applicable
Calibration Due Date_____________

Passed Verification:
Date of **Verification**_______________
Technician_______________________
Calibration Sticker: ☐ Yes
☐ No
☐ Not Applicable
Calibration Due Date_____________

Passed Verification:
Date of **Verification**_______________
Technician_______________________
Calibration Sticker: ☐ Yes
☐ No
☐ Not Applicable
Calibration Due Date_____________

Passed Verification:
Date of **Verification**_______________
Technician_______________________
Calibration Sticker: ☐ Yes
☐ No
☐ Not Applicable
Calibration Due Date_____________

Passed Verification:
Date of **Verification**_______________
Technician_______________________
Calibration Sticker: ☐ Yes
☐ No
☐ Not Applicable
Calibration Due Date_____________

Passed Verification:
Date of **Verification**_______________
Technician_______________________
Calibration Sticker: ☐ Yes
☐ No
☐ Not Applicable
Calibration Due Date_____________

Passed Verification:
Date of **Verification**_______________
Technician_______________________
Calibration Sticker: ☐ Yes
☐ No
☐ Not Applicable
Calibration Due Date_____________

Passed Verification:
Date of **Verification**_______________
Technician_______________________
Calibration Sticker: ☐ Yes
☐ No
☐ Not Applicable
Calibration Due Date_____________

Passed Verification:
Date of **Verification**_______________
Technician_______________________
Calibration Sticker: ☐ Yes
☐ No
☐ Not Applicable
Calibration Due Date_____________

Passed Verification:
Date of **Verification**_______________
Technician_______________________
Calibration Sticker: ☐ Yes
☐ No
☐ Not Applicable
Calibration Due Date_____________

Passed Verification:
Date of **Verification**_______________
Technician_______________________
Calibration Sticker: ☐ Yes
☐ No
☐ Not Applicable
Calibration Due Date_____________

Passed Verification:
Date of **Verification**_______________
Technician_______________________
Calibration Sticker: ☐ Yes
☐ No
☐ Not Applicable
Calibration Due Date_____________

VALIDATION

Example of an Autoclave IQ protocol:

AUTOCLAVE IQ

Objective

To demonstrate that the Autoclave manufactured by ____, model # _____ and accessories installed in building ____, room ___ conforms to the purchase specifications and the manufacturers literature, and to document the information that the equipment meets specifications.

Scope

For new installation, modification, replacement, or relocation of any critical component of the autoclave.

Responsibility

Supervisor of the Department where the autoclave is located is responsible for writing the protocol, supervising the performance of the IQ, verifying the data and writing the IQ report.

QA is responsible for approving the protocol and reviewing and approving the data and conclusions.

Systems/Equipment

Give a brief description of the autoclave indicating the manufacturer and model name/number, where it is located, what materials it will be sterilizing, any accessories that accompany it (e.g. carts) and provide a short description of how the autoclave functions.

Component List

Typical major components associated with autoclaves are:

> autoclave chamber, baffles, shell insulation, frame, doors, door seals, temperature detectors and probes (RTDs), temperature recording chart, safety valves, vacuum pump, side door motor, sterilization cart, pressure transmitters and gauges, microcomputer control, chamber high water sensor .

Procedure

Fill in the prepared checklists with the detailed mechanical and electrical specifications, drawings, etc. (as itemized in the IQ format) for each component as listed in the IQ format.

The individual component checklist includes a space to record the information plus any deviations found during the installation check.

Reporting

Responsible person verifies that the information is complete, prepares the Deviation Report and the Installation Qualification Report and, submits to QA for review and approval.

Example of an OQ protocol for an autoclave:

AUTOCLAVE OQ

Objective

To determine that the autoclave model # _____ , installed in building ___, room ___ operates according to specifications, to determine the heat /steam distribution in the jacket and empty chamber and to record all relevant information and data to demonstrate it functions as expected.

Scope

a) For new installation, modification, replacement, or relocation of any critical component of the autoclave.

b) If there is a contamination problem.

To be performed after the IQ has been completed and approved.

Responsibility

Supervisor of the Department where the autoclave is located is responsible for writing the protocol, supervising the performance of the OQ, verifying the data and writing the OQ report.

QA is responsible for approving the protocol and reviewing and approving the data and conclusions.

Equipment and Documents

Example of calibration instruments required are:

thermocouples, pressure calibrator, vacuum calibrator, temperature detectors and probes, timers, temperature bath, flow meters. (Certification methods should be referenced)

SOP# ___: Operation, Maintenance, and Calibration of the Autoclave

Training records for personnel operating and maintaining the autoclave.

The calibrating instruments must be certified before being used for calibrating the autoclave.

Procedure:

Typical critical parts of the autoclave to be calibrated are:

temperature sensors, pressure sensors, pressure gauges, pressure switches, pressure transmitters and input/output transmitter.

Typical alarm points to be checked on the autoclave are:

under or over temperature, evacuation too long, sterilization too long, vacuum system failure, door open, failure reading temperature or pressure or both, failure reading load, pressure in chamber with door unsealed, chamber flooded, insufficient vacuum level to perform leak test, low battery,

Proceed with the testing of the functions of the autoclave.

Operational Qualification for Autoclave

Approvals:

Preparer: Jason McMillan Date: 17JAN14
Reviewer: Dr. Maggie Bryans Date: 18JAN14

1. **Purpose:**
   1.1. Proper use of BTSure biological indicators to carry out an operational qualification of an autoclave.
2. **Scope:**
   2.1. To validate the ability of a laboratory autoclave to sterilize.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. BTSure Biological Indicator manufacturer instructions
   4.2. Autoclave SOP
   4.3. Incubator or water bath SOP
5. **Definitions:** N/A
6. **Precautions:**
   6.1. The contents of the BTSure biological indicator is extremely hot and under pressure after autoclaving. It may burst and cause burns. Allow at least 10 minutes to cool before handling it.
   6.2. Always wear the appropriate personnel protective equipment.
7. **Materials:**
   7.1. BTSure biological indicator unit(s)
   7.2. BTSure crusher
   7.3. autoclave
   7.4. incubator (55-60ºC) or water bath
   7.5. small beaker
   7.6. aluminum foil
   7.7. permanent marker
8. **Procedure:**
   8.1. Remove an appropriate number of BTSure units from the box.
   8.2. Remove one unit for each area of the autoclave to be tested and one additional unit to be used as a positive control (this one will not be placed in the autoclave).
   8.3. Label indicators with appropriate information.
   8.4. Place each unit in a small beaker so that they lay horizontally in the bottom of the beaker. Use a separate beaker for each BTSure unit.
   8.5. Cover the beakers with aluminum foil. **Note:** If a flash cycle is selected the goods should be unwrapped. If the come up time is less than one minute a three minute exposure cycle may have to be extended to four minutes to ensure the BI is killed.
   8.6. Place the beakers inside the autoclave except the positive control.

Operational Qualification for Autoclave

8.6.1. One of the beakers should be situated so that it is directly over or next to the drain. **Note:** The area surrounding the drain is the coolest part of the autoclave and considered to be the least effective area for sterilization.

8.7. Operate the autoclave per autoclave SOP.

8.8. Remove the beakers from the autoclave. Allow indicators to cool for at least 10 minutes.

8.9. Remove the biological indicators from the beakers.

8.10. Observe the color change (blue to black) of the chemical indicator on the BTSure Label. **Note:** Color change indicates exposure to steam. It does not indicate acceptable sterilization.

8.11. **Incubation**

8.11.1. Place an indicator into the crusher in an upright position and squeeze the crusher to break the glass ampoule. This will allow the strip to be immersed in the media.

8.11.2. Repeat the crushing with each additional indicator including the positive control.

8.11.3. Immediately place the indicators (including the positive control) into the incubator or water bath.

8.11.4. Incubate at 55 to 60°C for at least 48 hours.

8.12. **Interpretation**

8.12.1. During the incubation examine the indicators at regular intervals starting approximately at 4 hours, and again at approximately 8, 12, 18, and 24 hours for any change in color. Record results on the Autoclave Monitoring Form.

8.12.1.1. The positive control tube should change from purple media to yellow. If it does not, the study is invalid and needs to be repeated with a new lot of indicators.

8.12.1.2. The sample indicators should not change color. No color change (media remains purple) indicates adequate sterilization.

8.12.1.3. If the sample indicators turn yellow this indicates bacterial growth and an investigation will need to be conducted. This may include re-performing the autoclave cycle.

8.12.2. Report any indication of bacterial growth to instructor or lab manager.

8.12.3. Dispose all used BTSure tubes in a biohazard receptacle when finished.

Operational Qualification for Autoclave

9. Attachments:

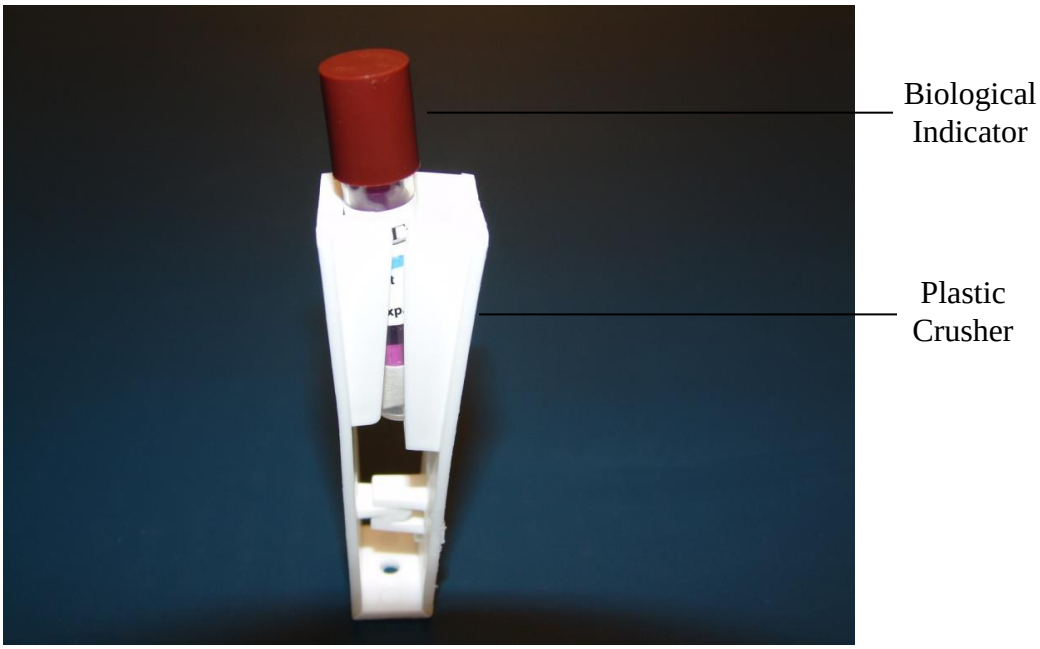


Figure 1: BTSure Biological Indicator in Plastic Crusher

10. History:

Revision Number	***Effective Date***	***Preparer***	***Description of Change***
0	25JAN06	Hope Townes and Kari Britt	Initial release
1	20JUL06	Bob O'Brien	Added photograph and disposal information.
2	20AUG06	Bob O'Brien	Added interval hours for examination.
3	12FEB07	Bob O'Brien	Updated date format.
4	11APR08	Bob O'Brien	College name change
5	15OCT09	Sonia Wallman	Additional information to purpose and scope.
6	10AUG10	Kari Britt	Added data table. Made formatting edits throughout.
7	17JAN14	Jason McMillan	Additional information to purpose and interpretation, college name change.

SOP: Tuttnauer 3850 ELV Autoclave Operation

Approvals:

Preparer:	John Buford	Date: 25NOV13
Reviewer:	Jason McMillan	Date: 25NOV13
Reviewer:	Maggie Bryans	Date: 26NOV13

1. **Purpose**
   1.1. Sterilize equipment, instruments, solutions, and biohazardous waste using the Tuttnauer 3850 ELV autoclave.
2. **Scope and Applicability**
   2.1. An autoclave sterilizes materials by means of pressurized steam, generally 15 PSI at 121ºC. Items to be autoclaved need to be heat and pressure stable. Items that are damaged by heat or pressure should be sterilized by other methods.
3. **Summary of Method**
   3.1. Prepare items to be sterilized.
   3.2. Load the autoclave.
   3.3. Select and run a sterilization program.
   3.4. Open the autoclave and allow load to cool.
   3.5. Perform a visual inspection and verify the sterilizing indicators.
4. **References**
   4.1. Tuttnauer Operation & Maintenance Manual, Laboratory Vertical Steamer Sterilizers models 2540, 3850, 3870 ELV and 2540, 3850, 3870 ELVC, Cat. No. MAN205-0350001EN Rev G.
5. **Definitions:**

Autoclave	A device used to sterilize equipment and supplies by subjecting them to high pressure steam.
Heat-labile	Susceptible to alteration or destruction by heat.

6. **Precautions**
   6.1. Steam from autoclave can cause serious burns. Verify that there is no pressure in the chamber before opening the autoclave door. Wear appropriate personal protective equipment such as eye protection and heat resistant gloves. Keep head and body parts well away from the area above the door. Open the door slowly to allow steam to escape.
   6.2. Items being autoclaved will be hot after the cycle completes. Wear appropriate personal protective equipment such as eye protection and heat resistant gloves when unloading the autoclave.
   6.3. When autoclaving liquids, never fill containers with more than 2/3 full.

SOP: Tuttnauer 3850 ELV Autoclave Operation

7. Responsibilities

7.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.

7.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

8. Equipment and Materials

8.1. Tuttnauer 3850 ELV autoclave
8.2. Safety glasses
8.3. Heat resistant gloves
8.4. Autoclave indicator tape
8.5. Aluminum foil
8.6. 8 liters laboratory grade water
8.7. Items to be sterilized

9. Procedure

9.1. ***Preparing items to be sterilized:***

9.1.1. Verify that all items are labeled appropriately (for example contents/prep date/initials).
9.1.2. Use only heat-proof glass containers for liquids, filled to no more than 2/3 capacity to avoid boil-overs.
9.1.3. Loosen all container caps to avoid breakage due to pressure.
9.1.4. Plug the opening of media flasks with gauze or cover loosely with aluminum foil.
9.1.5. Position empty containers upside down to prevent water from accumulating.
9.1.6. Do not mix liquid and non-liquid items in the same load.
9.1.7. Do not mix clean and biohazard items in the same load.
9.1.8. Place a small piece of autoclave indicator tape on each item.

9.2. ***Operating the autoclave:***

9.2.1. Open the autoclave door and remove the baskets from the autoclave.
9.2.2. Load the baskets with items to be sterilized, which have been prepared per section 9.1 above.
9.2.2.1. Position items so that steam with be able to penetrate.
9.2.2.2. Do not crowd or overload the baskets.
9.2.2.3. Do not exceed 20 liters of liquid or 20 kilograms (44 pounds) of instruments.
9.2.3. Inspect the door gasket. The gasket should be clean and smooth. Clean if necessary by wiping the gasket with a soft cloth using water and a mild soap solution.
9.2.4. Pour deionized water into the autoclave until the water level rises to the bottom grate.
9.2.5. Place the baskets into the autoclave.
9.2.6. Turn on the main power switch located at the bottom right corner of the keyboard panel.
9.2.7. Close and seal the autoclave door:
9.2.7.1. Carefully lower the door.

SOP: Tuttnauer 3850 ELV Autoclave Operation

9.2.7.2. Lift the tightening bolt into the vertical position, then rotate the handle clockwise until the bolt is hand tight. Do not over-tighten the bolt to avoid damaging the door gasket. Verify that the open door icon disappeared from the main screen and that "READY" is displayed indicating that the door is sealed.

9.2.8. Select one of the sterilization programs using the up and down arrow buttons on the top keypad. See Table 1 below for a description of each sterilization program.

9.2.9. Start the selected program using the START/STOP button on the top keypad. The door lock should click and the sterilization cycle begins.

9.2.10. The buzzer sounds and "CYCLE END" is displayed on the main screen at the end of the sterilization cycle.

9.2.11. Don appropriate personal protective equipment, including eye protection and heat resistant gloves.

9.2.12. Open the autoclave door:

(Caution: steam from autoclave can cause serious burns. Keep head and body parts well away from the area above the door.)

9.2.12.1. Verify that there is no pressure in the chamber by checking the pressure gauge located on the front.

9.2.12.2. Rotate the handle of the tightening bolt counter-clockwise until it spins loosely, then lower it to the horizontal position.

(Note: a safety device locks the door when the power is off, when a sterilization cycle is in progress, or when a sterilization cycle is stopped due to a failure or manual stop. If the tightening bolt will not rotate, ensure that the power is on and that a sterilization cycle in not in progress, then press the START/STOP key to cancel the door lock.)

9.2.12.3. Open the door just a crack. Allow steam to escape for at least 30 seconds.

9.2.12.4. Lift the door slowly to allow any remaining steam to escape.

9.2.13. Remove each of the loaded baskets by the handle from the autoclave carefully. Place loaded baskets in an undisturbed area. Do not touch the sterilized items.

9.2.14. Allow the loaded baskets to cool to room temperature.

9.2.15. Unload the baskets and perform a visual inspection to verify that the sterilizing indicators changed color and that the load is dry. Tighten container caps.

9.2.16. Replace the empty baskets in the autoclave and carefully lower the door. Leave the door unlatched to permit air circulation.

9.2.17. Turn off the main power switch located at the bottom right corner of the keyboard panel. Retain the paper printout in order to document the sterilization cycle in a lab notebook.

SOP: Tuttnauer 3850 ELV Autoclave Operation

10. Attachments:

Program	***Screen Icon***	***Sterilization Phase Time & Temp***	***Exhaust Phase***
Unwrapped Instruments	 (scissors)	4 minutes at 134ºC	Rapid
Unwrapped Delicate Instruments	 (empty flask)	20 minutes at 121ºC	Rapid
Liquid	 (full flask)	20 minutes at 121ºC	Slow

Table 1. Sterilization Programs
Tuttnauer 3850 ELV Manual

SOP: Tuttnauer 3850 ELV Autoclave Operation

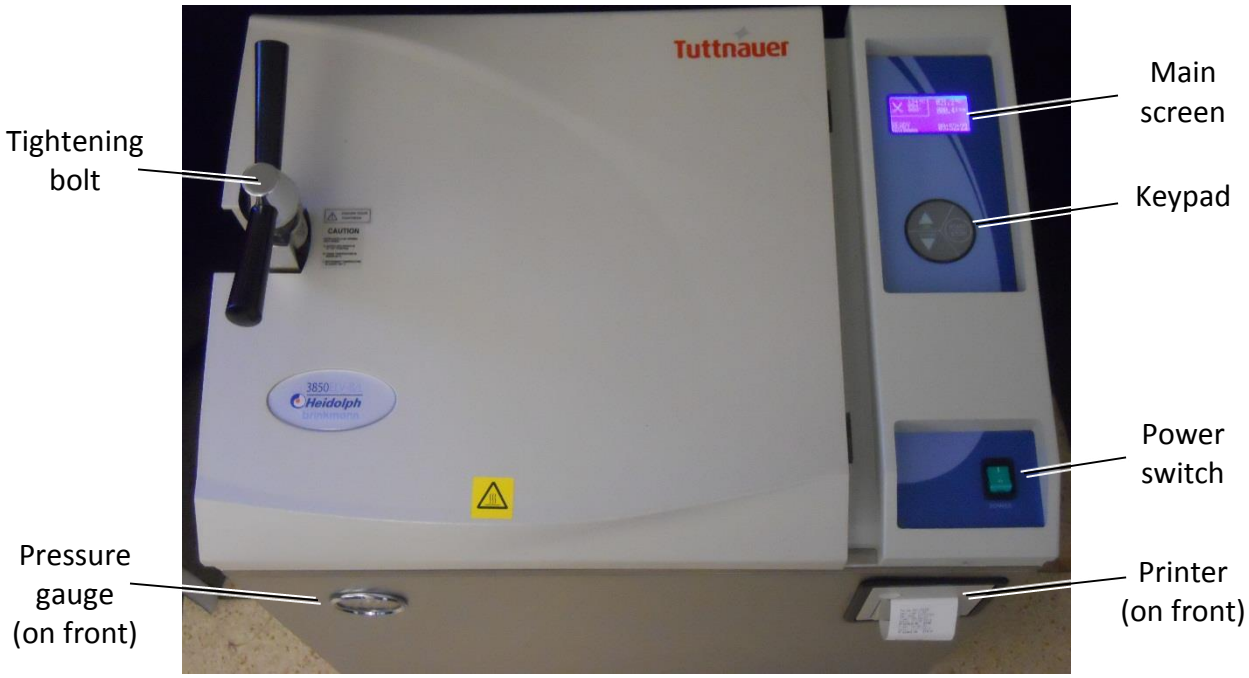


Figure 1. Tuttnauer 3850 ELV Autoclave (top view)
Tuttnauer 3850 ELV Manual

11. History

Revision Number	*Effective Date*	*Preparer*	*Description of Change*
0	27NOV13	John Buford	Initial release

SOP: Operation of Harvey Sterilemax Autoclave

Approvals

Preparer: Tim Kull Date: 23OCT12
Reviewer: Dr. Maggie Bryans Date: 23OCT12
Reviewer: Jason McMillan Date: 13JAN15

1. **Purpose**
   1.1. Operation of the Harvey Sterilemax autoclave.
2. **Scope and Applicability**
   2.1. Applies to the steam sterilization of a variety of materials.
3. **Summary of Method**
   3.1. Prepare the items to be sterilized
   3.2. Load the autoclave
   3.3. Select and sterilization program.
   3.4. Open the autoclave and allow the load to cool.
   3.5. Perform a visual inspection and verify the sterilizing indicators.
4. **References**
   4.1. Thermo-Scientific (Harvey) Sterilemax Operation Manual
5. **Definitions: N/A**
6. **Precautions**
   6.1. Do not attempt to open the door before display reads "cycle complete". Pressure in the chamber could cause the door to open with extreme force.
   6.2. Use caution when opening the door. Open door slowly and partially to protect yourself from steam releasing, boiling liquids and exploding flasks.
   6.3. Sterilizer trays and items being autoclaved will be very hot after cycle is complete. Always wear protective gloves when handling these items.
   6.4. When autoclaving liquids in bottles, beakers or tubes, never fill them more than 75%.
   6.5. Do not add water to the reservoir while cycle is running.
   6.6. Refer to pages 6-8 in operation manual for further precautions.
7. **Responsibilities**
   7.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   7.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
8. **Equipment and Materials**
   8.1. Item(s) to be sterilized
   8.2. Aluminum foil (if no lid applies)
   8.3. Autoclave tape
9. **Procedure**
   9.1. Turn on power switch under the right front corner.
   9.2. Check water level in the reservoir. The water level should be between the white arm at the bottom of the reservoir and the metal arm towards the top of the reservoir. If water is needed, add MilliQ water making sure to not overfill.

SOP: Operation of Harvey Sterilemax Autoclave

9.3. For items lacking a lid, for example over an open beaker or bottle with no lid, loosely place aluminum foil over the mouth of the vessel to create a lid.
9.4. If a bottle or test tube has a lid or cap, loosen the lid or cap.
9.5. Any container containing a liquid should be filled no more than 75%.
9.6. Place a piece of autoclave tape on item(s).
9.7. Load the sterilizer chamber, with your item(s), onto the perforated tray.
9.8. Close the door completely and tighten the lever clockwise.
9.9. Select process cycle by first pressing the start button on touch pad.
9.9.1. Choose cycle (refer to page 12 of manual).
9.9.2. Press start again.
9.10. After cycle is complete autoclave will beep and the screen will read "cycle complete" and "open door". To open the door you must first untighten the lever counter clockwise then open the door release on the bottom left of the door. DO THIS WITH CAUTION BECAUSE OF STEAM RELEASING OUT!!
9.11. To retrieve your items out of the autoclave when cycle is finished, be sure to be wearing protective gloves.

10. Attachments: N/A

11. History

Revision Number	***Effective Date***	***Preparer***	***Description of Change***
0	24OCT12	Tim Kull	Initial release
1	13JAN15	Jason McMillan	Format change and minor revisions

FORM: Autoclave Monitoring

Autoclave Information

Name and Description:_____

Model:_____

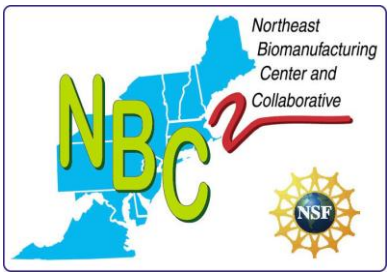
Serial Number:_____

Biological Indicator Test Results

Date	Time	Hours since incubation began	Color of media inside indicator	Comments	Initials

Monitoring Results:	**Failed Monitoring:**
Dates Monitoring Protocol Performed: _____ Monitoring Protocol Initiator: _____ Pass: □Yes □ No	Reason for Failure:_____ _____ _____ Date Out of Service:_____ Technician:_____

ENVIRONMENTAL HEALTH & SAFETY



Northeast Biomanufacturing Center and Collaborative

Hazard Communication Program (HCP)

NBC-HCP-000

Date Issued: **06/27/06**
Date Revised: **05/30/07**

APPLICIPABLE STANDARD: 29CFR1910.1200
OSHA's Hazard Communication Standard

Document Contents

SECTION CONTENT
A Application
B Purpose
C Definitions
D Scope
E Chemical Inventory List
F MSDS
G Labeling
H Training
I Outside Contractors

Section A

Application

The Northeast Biomanufacturing Center and Collaborative (NBC^2), under OSHA's Hazard Communication Standard (HCS), requires chemical manufacturers or importers to assess the hazards of all chemicals they produce or import and transmit that information to affected employers and employees. The standard requires the Center to provide employee information about the potential of hazardous chemical exposure under normal use conditions or in a foreseeable emergency; and the transmittal of this information by means of a comprehensive Hazard Communication Program which includes container labeling and other forms of warning, Material Safety Data Sheets, and employee training.

In order to comply with the Hazard Communication Standard, this Hazard Communication Program has been established for NBC^2. All schools, colleges, and administrative offices operating within the center are incorporated within this program.

Primary responsibility for compliance with the Hazard Communication Program lies with the individual institution, laboratory, or facility operating within the center. Each dean, director, chair, and lab supervisor is responsible for safety performance and hazard communication within their respective institutions and labs. The Environmental Health and Safety Department

(EH&S) is responsible development and coordination of policies and procedures. EH&S also provides technical assistance in establishing procedures and monitoring performance in activities involving the Hazard Communication Program.

NBC^2 is committed to creating, maintaining and promoting a safe and healthful environment for all associated individuals including students, faculty, staff employees, hospital patients, and visitors. A critical component of NBC^2's environmental health and safety commitment is integrating information concerning chemical hazards into all academic and operational activities by means of this Hazard Communication Program.

This program outlines the definitions, procedures and training requirements to be utilized by NBC^2 employees and trainees to understand and comply with the Hazard Communication Standard. It is the duty of each employee to become familiar with the contents of this program and ensure compliance with its procedures. Supervisors and instructors shall ensure that employees and trainees understand the details of this program and ensure that employees receive the proper training. Supervisors and instructors are also responsible for maintaining records of this training. These records must be current and readily available for review.

ASSOCIATED DOCUMENTS

NBC-HCP-001: Safety self-audit
NBC-HCP-002: Guidance Document

Section B

PURPOSE

- The NBC2 Hazard Communication Program (HCP) was developed to:
- Inform employees of the hazards associated with chemicals in the workplace.
- Ensure safe use, handling and disposal of hazardous chemicals in the workplace.
- Comply with the Occupational Safety and Health Administration's (OSHA) Hazard Communication Standard (29 CFR 1910.1200)
- A successful Hazard Communication Program will reduce potential incidents of chemical source illnesses and injuries.

Section C

Definitions

Chemical means any element, chemical compound or mixture of elements and or/ compounds.

Chemical manufacturer means an employer with a workplace where chemical(s) are produced for use or distribution.

Chemical name means the scientific designation of a chemical in accordance with the nomenclature system developed by the International Union of Pure and Applied Chemistry (IUPAC) or the Chemical Abstracts Service (CAS) rules nomenclature, or a name, which clearly identify the chemical for the purpose of conducting a hazard evaluation.

Combustible liquid means any liquid having a flashpoint at or above 100 degree F, but below 200 degree F, except any mixture having components with flashpoints of 200 degree F, or higher, the total volume of which make up 99% or more of the total volume of the mixture.

Common name means any designation or identification such as code name, code number, trade name, brand name or generic name used to identify a chemical other than by its chemical name.

Container means any bag, barrel, bottle, box, can, cylinder, drum, reaction vessel, storage tank, or the like that contains a hazardous chemical.

Distributor means a business, other than a chemical manufacturer or importer, which supplies hazardous chemicals to other distributors or to employers.

Employee means a worker who may be exposed to hazardous chemicals under normal operating conditions or in foreseeable emergencies.

Employer means a person engaged in a business where chemicals are either used, distributed, or are produced for use or distribution, including a contractor or subcontractor.

Explosive means a chemical that causes a sudden, almost instantaneous release of pressure, gas, and heat when subjected to sudden shock, pressure, or high temperature.

Exposure or exposed means that an employee is subjected in the coarse of employment to a chemical that is a physical or health hazard, and includes potential exposure.

Flammable means a chemical that falls into one of the flowing categories:

- "Aerosol, flammable" means an aerosol that, when tested by the method described in 16 CFR 1500.45 yields a flame projection exceeding 18 inches at full valve opening, or a flashback at any degree of valve opening
- "Flammable Gas" means a gas that, at ambient temperature and pressure, forms a flammable mixture with air at a concentration of thirteen percent by volume or less; or a gas that at ambient temperature and pressure, forms a range of flammable mixtures with air wider than twelve percent by volume, regardless of the lower limit
- "Flammable Liquid" means any liquid having a flashpoint below 100 degree F, except any mixture having components with flashpoints of 100 degree F or higher, the total of which make up 99% or more of the total volume of the mixture
- "Flammable Solid" means a solid, other than a blasting agent or explosive as defined in CFR 1971.109, that is liable to cause fire through friction, absorption of moisture, spontaneous chemical change, or retained heat from manufacturing or processing, or which can be ignited readily and when ignited burns so vigorously and persistently as to create a serious hazard

Flashpoint means the minimum temperature at which a liquid gives off a vapor.

Foreseeable emergency means any potential occurrence such as, but not limited to, equipment failure, rupture of containers, or failure of control equipment, which could result in an uncontrolled release of a hazardous chemical into the workplace.

Hazardous chemical means any chemical, which is a physical hazard or a health hazard.

Hazard warning means any words, pictures, symbols, or combination thereof appearing on a label or other appropriate form of warning which convey the specific physical and health hazard including target organ effects, of the chemicals in the containers.

Health hazard means a chemical for which there is statistically significant evidence based on at least one study conducted in accordance with the established scientific principles that acute or chronic health effects may occur in exposed employees.

Identity means any chemical or common name, which is indicated on the material safety data sheet (MSDS) for the chemical.

Immediate use means that the hazardous chemical will be under the control of and used only by the person who transfers it from a labeled container and only within the work shift it is transferred.

Label means any written, printed, or graphic material displayed on or affixed to containers of hazardous chemicals

Material Safety Data Sheets (MSDS) means written or printed material concerning a hazardous chemical.

Mixture means any combination of two or more chemicals if the combination is not, in whole or in part, the result of a chemical reaction.

Oxidizer means a chemical other than a blasting agent or explosive as defined in CFR 1910.109(a), that initiates or promotes combustion on other materials, thereby causing fire either of itself or through the release of oxygen or other gases.

Physical hazard means a chemical for which there is scientifically valid evidence that it is combustible liquid, compressed gas, explosive, flammable, an organic peroxide, an oxidizer, pyrophoric, unstable or water-reactive.

Produce means to manufacture, process, formulate, blend, extract, generate, emit, or repackage.

Responsible party means someone who can provide additional information on the hazardous chemical and appropriate emergency procedures, if necessary.

Specific chemical identity means the chemical name, Chemical Abstracts Service (CAS) Registry Number, or any other information that reveals the precise chemical designation of the substance.

Unstable (reactive) means a chemical which in the pure state, or as produced or transported, will vigorously polymerize, decompose, condense, or will become self-reactive under conditions of shocks, pressure, or temperature.

Use means to package, handle, react, emit, extract, generate as a byproduct, or transfer**.**

Water-reactive means a chemical that reacts with water to release a gas that is either flammable or presents a health hazard.

Work area means a room or defined space in a workplace where hazardous chemicals are produced or used, and where employees are present.

Workplace means an establishment, job site, or project, at one geographical location containing one or more work areas.

Section D

SCOPE

This program applies to:

- All departments which use or store chemicals and
- All chemicals used by employees under normal conditions of work or in foreseeable emergencies.

EXCEPTION: This program does not apply to research laboratories. These facilities are subject to Chemical Hygiene Plan requirements under the OSHA Laboratory Standard, 29 CFR 1910.1450. For more information, contact your Chemical Hygiene Officer.

Section E

CHEMICAL INVENTORY LIST

In Appendix A of this program is a blank Chemical Inventory List Form. All chemicals being used in this department and for which there is an MSDS in the binder are to be listed on this Inventory form. The completed, current Inventory is to reside in the MSDS binder kept in this department.

Procedure for maintaining the inventory list:

1. When a chemical product is received at this department, retrieve the Program binder.

2. Check the Inventory List for the name of the chemical product as it appears on the MSDS accompanying the shipment.

3. If the product is not listed, add it to the list.

Section F

MSDS

An MSDS must be kept on file for all chemicals on the Inventory list. MSDS's are designed to provide the information needed to handle chemicals safely. MSDS's may differ somewhat in format and content, however all should contain the following:

1. Substance identification - names, synonyms, manufacturer contact information, and index numbers.
2. List of active and inert ingredients - components and contaminants.
3. Exposure limits - ACGIH, TLV, OSHA PEL, etc.
4. Physical data – boiling, melting points, vapor pressure, evaporation rate, specific gravity or density, water solubility, physical description.
5. Fire and explosion data – LEL, flashpoint, flammability, class of hazardous atmosphere, firefighting media and methods, including fire extinguishers, etc.

6. Transportation requirements, if any
7. Toxicity and health hazard data - including target organ, specific acute and chronic health effects, potential cancer risk, first aid and emergency medicine.
8. Storage and disposal - including reporting requirements
9. Spill and emergency response procedures.
10. Measures to protect employees including personal protective equipment, safety shower and eyewash, etc.

Please contact EHS or the Chemical Hygiene Officer if an MSDS appears to be inadequate, illegible, out-of-date or incomplete.

MSDS Procedures

1. Obtain and label one or more three ring binders and label "MSDS's". Place all old and new MSDS's in the binder(s). File MSDS's alphabetically and by use, location or other suitable category (this may already have been done in many departments).
2. Check all deliveries of chemicals for the MSDS(s). An MSDS should accompany the first shipment of all new or re-formulated chemicals.
3. When a chemical is received with an MSDS, place it in the binder and add the product name to the Chemical Inventory List. Discard any old or out-of-date MSDS for the same or similar product that is no longer in stock.
4. If a chemical is received without an MSDS, check the MSDS binder to determine if it already contains the MSDS. If not, immediately request one from the supplier. Store the chemical separately, label "DO NOT USE" and do not use until the MSDS is received.
5. MSDSs can also be obtained from vendor web sites.
6. Inform all employees of the location of the MSDS binder.

MSDS Location_____

Use of MSDS in Exposure Incidents

If an employee is exposed to a chemical and the exposure results in an illness or injury that requires treatment by medical personnel:

1. Ensure that medical personnel see the individual immediately.

2. Provide a copy of the MSDS to the medical personnel involved. Along with the MSDS provide any additional information you have on the chemical and when, where and how it was used.

Section G

LABELING

Primary container label contents - Labels on all primary containers must include:

1. The identity of the chemical - common name &/or chemical name.
2. A hazard warning – such as "Caution, Warning, Flammable, Toxic", etc.
3. The name and address of the manufacturer.
4. Chemical hazard ratings for health, fire reactivity (HMIS)
5. Target organs that may be affected by chronic health hazards

Anytime a container contains the information listed above, an additional HMIS label will not be required. We will accept the manufactures original label if it meets this criteria.

Primary container label procedure:

1. When chemicals are received, check all containers to ensure that the product label meets the requirements outlined above.
2. With each chemical shipment the purchasing agent or his/her designee will check all containers to ensure that the condition is safe and that all labels meet the requirements outlined in this program. Do not accept unsafe containers or improperly labeled containers.

A secondary container is any container other than the one in which the chemical was received from the supplier. Secondary container labels will contain the same information as labels for primary containers. All labeling information can be obtained from the original container, or the MSDS for the product. Label secondary containers if:

- More than one employee uses the container.
- The container is used longer than one shift or left unattended in the work area.
- Labels on containers not containing original product will be removed and re-labeled
- Any portable containers used to store, transport or transfer chemicals which hold a sufficient amount to present a physical or health hazard must be labeled

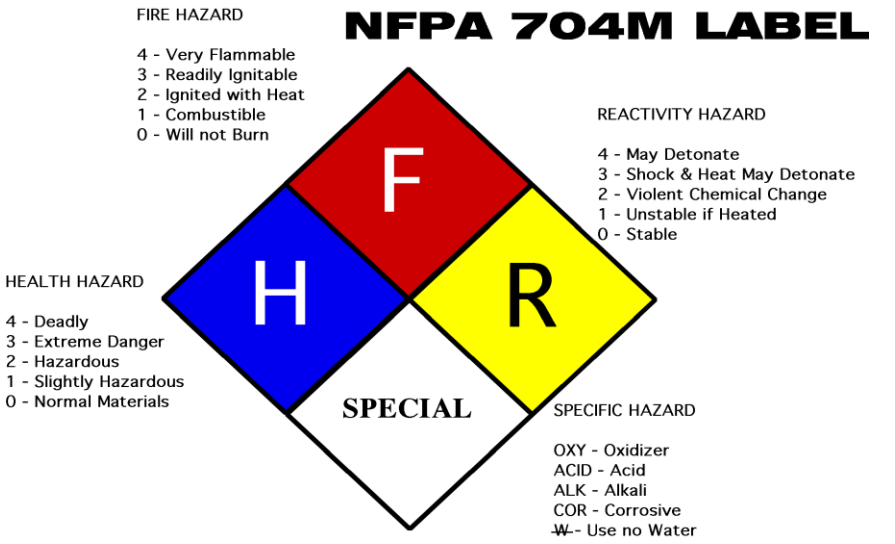
It is not necessary to label the secondary container if:

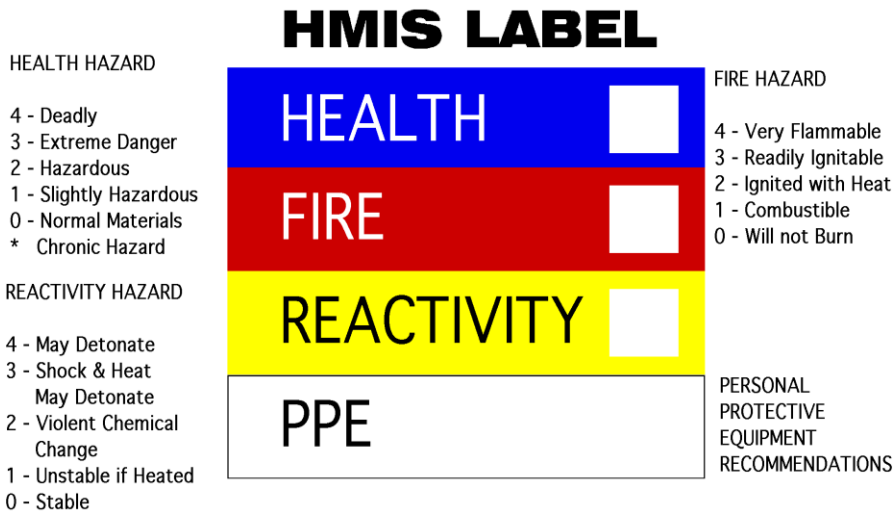
- one employee uses the chemical without exposing others, and
- returns the contents to the original container or disposes of the rest of it.

We will be using the National Fire Protection Association (NFPA) or Hazardous Material Information System (HMIS) to label items that are not correctly labeled or items that do not have labels. This includes numerical ratings for the acute health, flammability and reactivity hazard, the assignments of a personal protective equipment index and the designation of chronic health hazards

The hazard communication portion of the NFPA communicate information on:

1. Chemical identify – common names and code numbers
2. Degree of Acute Health, Flammability and Reactivity hazards – numerical rating
3. Proper Personal Protective Equipment – pictograms
4. Chronic Health Hazards





Section H

TRAINING

Training Matrix - Use this table to determine training requirements. These requirements apply to all employees who will use hazardous chemicals in the course of their job duties. Please contact NBC2 for assistance with your training needs

When	Content	Training methods
Initially, prior to assignment to work	• Details of this program • OSHA requirements • Physical, health hazards of exposure to hazardous chemicals • How to use MSDS's, labels (and other warnings if any) • Location of MSDS's, inventory list and copies of this program. • How to detect presence or release of hazardous chemicals. • Measures to protect employees including safe work practices, PPE, and emergency procedures	Classroom type training. Video, other AV and interactive media are useful for this application
Upon introduction of	• Physical, health hazards of exposure to hazardous chemicals	Safety meeting, job, facility or task orientation

new hazards, (new chemicals, new tasks, etc.)	• Measures to protect employees including safe work practices, PPE, and emergency procedures	
Upon assignment to non-routine tasks	• Physical, health hazards of exposure to hazardous chemicals • Measures to protect employees including safe work practices, PPE, and emergency procedures	Safety meeting should include walkthrough and task orientation.

Keep these training records:

- Date of training.
- Name and job title of trainer.
- Names of the trainees.
- Training topics.
- Other pertinent information to substantiate the training

Note: Please see Training Record Form, Appendix B.

Section I

OUTSIDE CONTRACTORS

During a pre-job walkthrough or meeting:

- Inform contractors of any hazards in the work area that their employees may encounter during the term of the contract.

During the term of the contract, observe work practices to ensure that contractors are complying with OSHA. Contractors are required to observe the following guidelines (these guidelines apply to all sub-contractors also):

- Establish and enforce safe work practices.
- Comply with all applicable OSHA requirements
- Inform department in advance of all hazardous materials to be used during a project. Inform building occupants upon request by occupants or any employee.
- Supply a copy of all MSDS's for those materials upon request.
- Verify that each container used is labeled in accordance with this HCP.
- A copy of the NBC2 Hazard Communication Program will be available

Appendix A

Chemical Inventory List

Appendix A
Chemical Inventory List

Chemical Name	Manufacturer/supplier

Appendix B

Employee Training Record

Employee Training Record

Supervisor:	Department:
Unit:	Location:
Date Training Developed:	Training Program Revision Dates:

Employee Name	Job Title	General Haz Com Training (most recent date)	Unit or Dept Specific Haz Com Training (most recent date)

Lab, Clinic, or Unit Specific Hazard Communication Training Provided: List the specific training provided by the PI or supervisor to the individuals listed above. Provide separate descriptions as necessary.
1. Explanation of the Completed Unit Specific Haz Com Plan:
2. Chemical Inventory and specific hazardous chemical each employee may encounter
3. Location and availability of MSDSs; explanation of hazards associated with chemicals or groups of chemicals
4. Labeling system used by unit
5. Specific safety training for each person's duties including specific equipment and its use: **Specific PPE- the types used, its use, location, and limitations**:

6. Procedures for non-routine tasks and emergencies
7. Procedures when working with contractors

Written Program

Yes	No	
❑	❑	Department or laboratory has a copy of the Hazard Communication Program.
❑	❑	HazCom program Administrator identified.
❑	❑	Written HazCom program displayed and accessible to employees at all times.
❑	❑	All employees informed of HazCom program location.
❑	❑	HazCom Program reviewed annually

Chemical Inventory

Yes	No	
❑	❑	All chemicals located in the workplace are listed on the chemical inventory.
❑	❑	All employees have access to the chemical inventory list.
❑	❑	Individual has been designated to update Chemical Inventory list.
❑	❑	List is accurately updated when chemical product is received; chemical inventory is checked to ensure chemical is recorded.

MSDS

Yes	No	
❑	❑	MSDS location identified and ALL employees are informed of location.
❑	❑	MSDS arranged in an orderly fashion to ensure ease of location.
❑	❑	Each chemical present has an MSDS.
❑	❑	Employee designated to ensure MSDS are maintained in accordance with 29CFR1910.1200.
❑	❑	MSDS for chemicals no longer being used are kept in a separate location.
❑	❑	MSDS are legible and current.
❑	❑	MSDS for chemicals involved in an exposure incident are kept and present for the 30-year requirement.
❑	❑	MSDS meets informational requirements in accordance with 29CFR1910.1200.

Labeling

Yes	No	
❑	❑	Primary containers contain appropriate labeling information, (Chemical name, name and address of manufacturer, and appropriate warning info).
❑	❑	Secondary containers contain appropriate labeling information.
❑	❑	Employee designated to ensure labels are correct.
❑	❑	Primary and Secondary labels are updated and legible

Training

Yes No

- ❑ ❑ All employees receive training in Hazard Communication in accordance with the NBC2 Hazard communication program and 29CFR1910.1200.
  - Initially, prior to assignment to work
  - Upon introduction of new hazards (new chemicals, new tasks, etc.)
  - Upon assignment to non-routine tasks
- ❑ ❑ Training records kept on file by Supervisor / Foreman.
- ❑ ❑ Employee's know what to do in case of an emergency

Contractors

Yes No

- ❑ ❑ Outside contractors are informed of NBC2 Hazard Communication policy.
- ❑ ❑ Outside contractors are informed of hazardous chemicals to which they may potentially be exposed.
- ❑ ❑ Outside contractors inform employees of Hazardous Chemicals brought onto property.

Comments:_____
_____
_____

Date:_____
Evaluator:_____

THIS IS A SAMPLE OSHA MSDS TEMPLATE

Material Safety Data Sheet
May be used to comply with OSHA's Hazard Communication Standard, 29 CFR 1910 1200. Standard must be consulted for specific requirements.

U.S. Department of Labor
Occupational Safety and Health Administration
(Non-Mandatory Form)
Form Approved
OMB No. 1218-0072

IDENTITY *(as Used on Label and List)*

Note: Blank spaces are not permitted. If any item is not applicable or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's name	Emergency Telephone Number
Address *(Number, Street, City, State and ZIP Code)*	Telephone Number for Information
	Date Prepared
	Signature of Preparer *(optional)*

Section II—Hazardous Ingredients/Identity Information

Hazardous Components (Specific Chemical Identity, Common Name(s))	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (optional)

Section III—Physical/Chemical Characteristics

Boiling Point		Specific Gravity (H_20 = 1)	
Vapor Pressure (mm Hg)		Melting Point	
Vapor Density (AIR = 1)		Evaporation Rate (Butyl Acetate = 1)	

Solubility in Water

Appearance and Odor

Section IV—Fire and Explosion Hazard Data

Flash Point (Method Used)	Flammable Limits	LEL	UEL

Extinguishing Media

Special Fire Fighting Procedures

Unusual Fire and Explosion Hazards

(Reproduce locally) OSHA 174 Sept. 1985

Section V—Reactivity Data

Stability	Unstable		Conditions to Avoid
	Stable		

Incompatibility *(Materials to Avoid)*

Hazardous Decomposition or Byproducts

Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur		

Section VI—Health Hazard Data

Route(s) of Entry	Inhalation?	Skin?	Ingestion?

Health Hazards *(Acute and Chronic)*

Carcinogenicity	NTP?	IARC Monographs?	OSHA Regulated?

Signs and Symptoms of Exposure

Medical Conditions
Generally Aggravated by Exposure

Emergency and First Aid Procedures

Section VII—Precautions for Safe Handling and Use

Steps to Be Taken in Case Material Is Released or Spilled

Waste Disposal Method

Precautions to Be Taken in Handling and Storing

Other Precautions

Section VII—Control Measures

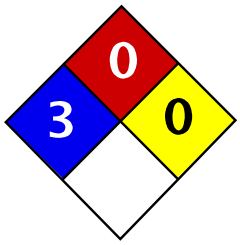
Respiratory Protection *(Specify Type)*

Ventilation	Local Exhaust	Special
	Mechanical *(General)*	Other

Protective Gloves	Eye Protection

Other Protective Clothing or Equipment

Work/Hygienic Practices



Health	3
Fire	0
Reactivity	0
Personal Protection	J

Material Safety Data Sheet
Potassium cyanide MSDS

Section 1: Chemical Product and Company Identification

Product Name: Potassium cyanide

Catalog Codes: SLP3853

CAS#: 151-50-8

RTECS: TS8750000

TSCA: TSCA 8(b) inventory: Potassium cyanide

CI#: Not available.

Synonym:

Chemical Name: Potassium Cyanide

Chemical Formula: KCN

Contact Information:

Sciencelab.com, Inc.
14025 Smith Rd.
Houston, Texas 77396

US Sales: **1-800-901-7247**
International Sales: **1-281-441-4400**

Order Online: ScienceLab.com

CHEMTREC (24HR Emergency Telephone), call:
1-800-424-9300

International CHEMTREC, call: 1-703-527-3887

For non-emergency assistance, call: 1-281-441-4400

Section 2: Composition and Information on Ingredients

Composition:

Name	CAS #	% by Weight
Potassium cyanide	151-50-8	100

Toxicological Data on Ingredients: Potassium cyanide: ORAL (LD50): Acute: 5 mg/kg [Rabbit]. 8.5 mg/kg [Mouse]. 5 mg/kg [Rat].

Section 3: Hazards Identification

Potential Acute Health Effects:
Very hazardous in case of skin contact (irritant, permeator), of eye contact (irritant), of ingestion, of inhalation. Corrosive to eyes and skin. The amount of tissue damage depends on length of contact. Eye contact can result in corneal damage or blindness. Skin contact can produce inflammation and blistering. Inhalation of dust will produce irritation to gastro-intestinal or respiratory tract, characterized by burning, sneezing and coughing. Severe over-exposure can produce lung damage, choking, unconsciousness or death. Inflammation of the eye is characterized by redness, watering, and itching. Skin inflammation is characterized by itching, scaling, reddening, or, occasionally, blistering.

Potential Chronic Health Effects:
CARCINOGENIC EFFECTS: Not available.
MUTAGENIC EFFECTS: Mutagenic for mammalian somatic cells.
TERATOGENIC EFFECTS: Not available.
DEVELOPMENTAL TOXICITY: Not available.

The substance is toxic to blood, liver.
The substance may be toxic to cardiovascular system, upper respiratory tract, Urinary system, central nervous system (CNS).
Repeated or prolonged exposure to the substance can produce target organs damage. Repeated exposure of the eyes to a low level of dust can produce eye irritation. Repeated skin exposure can produce local skin destruction, or dermatitis. Repeated inhalation of dust can produce varying degree of respiratory irritation or lung damage. Repeated exposure to a highly toxic material may produce general deterioration of health by an accumulation in one or many human organs.

Section 4: First Aid Measures

Eye Contact:
Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Cold water may be used. Get medical attention immediately.

Skin Contact:
In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Cover the irritated skin with an emollient. Cold water may be used.Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention immediately.

Serious Skin Contact:
Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek immediate medical attention.

Inhalation:
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.

Serious Inhalation:
Evacuate the victim to a safe area as soon as possible. Loosen tight clothing such as a collar, tie, belt or waistband. If breathing is difficult, administer oxygen. If the victim is not breathing, perform mouth-to-mouth resuscitation. WARNING: It may be hazardous to the person providing aid to give mouth-to-mouth resuscitation when the inhaled material is toxic, infectious or corrosive. Seek immediate medical attention.

Ingestion:
If swallowed, do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention immediately.

Serious Ingestion: Not available.

Section 5: Fire and Explosion Data

Flammability of the Product: Non-flammable.

Auto-Ignition Temperature: Not applicable.

Flash Points: Not applicable.

Flammable Limits: Not applicable.

Products of Combustion: Not available.

Fire Hazards in Presence of Various Substances: Not applicable.

Explosion Hazards in Presence of Various Substances:
Risks of explosion of the product in presence of mechanical impact: Not available.
Risks of explosion of the product in presence of static discharge: Not available.
Explosive in presence of oxidizing materials.

Fire Fighting Media and Instructions: Not applicable.

Special Remarks on Fire Hazards: Contact with acids or acid salts causes immediate formation of toxic and flammable hydrogen cyanide gas.

Special Remarks on Explosion Hazards:
Chlorates + potassium cyanide explode when heated.
Potassium cyanide + nitrites may cause explosion.
Nitrogen trichloride explodes on contact with potassium cyanide.
Potassium cyanide + hydrogen cyanide is a frictioin and impact-sensitive explosive and may initiate detonation of liquid hydrogen cyanide.
Mercuric nitrate + potassium cyanide explodes when heated and contained in narrow iignition tubes.
Perchloryl fluoride + potassium cyanide causes an explosive reaction at 100-300 C.
Potassium cyanide + ammoniacal silver, following heating, shock or standing can cause an explosion.
Heating of potassium cyanide & chromium tetraoxide can cause an explosion.
Mixtures of metal cyanides with metal chlorates, perchlorates, or nitrates causes a violent explosion.

Section 6: Accidental Release Measures

Small Spill:
Use appropriate tools to put the spilled solid in a convenient waste disposal container. If necessary: Neutralize the residue with a dilute solution of acetic acid.

Large Spill:
Corrosive solid. Poisonous solid.
Stop leak if without risk. Do not get water inside container. Do not touch spilled material. Use water spray to reduce vapors. Prevent entry into sewers, basements or confined areas; dike if needed. Call for assistance on disposal. Neutralize the residue with a dilute solution of acetic acid. Be careful that the product is not present at a concentration level above TLV. Check TLV on the MSDS and with local authorities.

Section 7: Handling and Storage

Precautions:
Keep locked up.. Keep container dry. Do not ingest. Do not breathe dust. Never add water to this product. In case of insufficient ventilation, wear suitable respiratory equipment. If ingested, seek medical advice immediately and show the container or the label. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents.

Storage:
Moisture Sensitive. Light Sensitive. Protect from light. Keep container tightly closed. Keep container in a cool, well-ventilated area. Do not store above 24°C (75.2°F).

Section 8: Exposure Controls/Personal Protection

Engineering Controls:
Use process enclosures, local exhaust ventilation, or other engineering controls to keep airborne levels below recommended exposure limits. If user operations generate dust, fume or mist, use ventilation to keep exposure to airborne contaminants below the exposure limit.

Personal Protection:
Splash goggles. Synthetic apron. Vapor and dust respirator. Be sure to use an approved/certified respirator or equivalent. Gloves.

Personal Protection in Case of a Large Spill:
Splash goggles. Full suit. Vapor and dust respirator. Boots. Gloves. A self contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

Exposure Limits:
STEL: 5 (mg/m3) from ACGIH (TLV) [United States]
CEIL: 0.7 from NIOSH [United States]
CEIL: 5 (mg/m3) from NIOSH [United States]
Consult local authorities for acceptable exposure limits.

Section 9: Physical and Chemical Properties

Physical state and appearance:
Solid. (Crystalline or Granular solid. Deliquescent solid.)

Odor:
Almond-like. Like bitter almonds. Odor of hydrogen cyanide (Slight.)

Taste: Not available.

Molecular Weight: 65.11 g/mole

Color: White.

pH (1% soln/water): 11 [Basic.]

Boiling Point: 1625°C (2957°F)

Melting Point: 634.5°C (1174.1°F)

Critical Temperature: Not available.

Specific Gravity: 1.553 (Water = 1)

Vapor Pressure: Not applicable.

Vapor Density: Not available.

Volatility: Not available.

Odor Threshold: Not available.

Water/Oil Dist. Coeff.: Not available.

Ionicity (in Water): Not available.

Dispersion Properties: See solubility in water, methanol.

Solubility:
Easily soluble in hot water.
Soluble in cold water.
Partially soluble in methanol.
Very slightly soluble in ethanol.
Partially soluble in glycerol, hydroxylamine, and liquid ammonia.
Very soluble in formamide

Section 10: Stability and Reactivity Data

Stability: The product is stable.

Instability Temperature: Not available.

Conditions of Instability: Incompatible materials, water, moisture, light,air

Incompatibility with various substances:
Highly reactive with oxidizing agents.
Reactive with acids.

Corrosivity: Non-corrosive in presence of glass.

Special Remarks on Reactivity:
Moisture sensitive.
Air Sensitive.
Deliquescent.
Protect from light.
Reacts with water or any acid releasing hydrogen cyanide.
Toxic gases and vapors (such as hydrogen cyanide and carbon monoxide) may be released when potassium cyanide decomposes.
Incompatible with acids, acid syrups, alkaloids, chloral hydrate, iodine, metallic salts, permanganates, chlorates, peroxides.
Potassium cyanide may react with carbon dioxide in ordinary air to form toxic hydrogen cyanide gas.
Potasssium cyanide is readily oxidized by heating to potassium cyanate in presence of oxygen or easily reduced oxides.

Special Remarks on Corrosivity: Not available.

Polymerization: Will not occur.

Section 11: Toxicological Information

Routes of Entry: Absorbed through skin. Dermal contact. Inhalation. Ingestion.

Toxicity to Animals: Acute oral toxicity (LD50): 5 mg/kg [Rat].

Chronic Effects on Humans:
MUTAGENIC EFFECTS: Mutagenic for mammalian somatic cells.
Causes damage to the following organs: blood, liver.
May cause damage to the following organs: cardiovascular system, upper respiratory tract, Urinary system, central nervous system (CNS).

Other Toxic Effects on Humans: Very hazardous in case of skin contact (irritant, permeator), of ingestion, of inhalation.

Special Remarks on Toxicity to Animals: Not available.

Special Remarks on Chronic Effects on Humans:
May cause adverse reproductive effects (female fertility and fetotoxicity).
May affect genetic material.

Special Remarks on other Toxic Effects on Humans:
Acute Potential Health Effects:
Skin: May be fatal if absorbed through skin. Causes skin irritation and possible burns especially if the skin is wet or moist. May be absorbed through skin and cause symptoms similar to those described for ingestion.
Eyes: Causes eye irritation and possible eye burns.
Inhalation: May be fatal if inhaled. Causes respiratory tract and mucous membrane irritation. Inhalation of high concentrations may cause central nervous system effects similar to those described for ingestion.
Ingestion: May be fatal if swallowed. Causes severe gastrointestinal tract irritation with nausea, vomiting and possible burns. May cause tissue anoxia. May affect behavior/Central Nervous system, Metabolism, cardiovascular system, respiratory system, blood, respiration. Symptoms of cyanide poisoning may include flushing, nausea, vomiting, palpitations, tachycardia, hypotension, hypertension, increased pulse rate, arrhythmias, heart conduction defects, hypernea, headache, dizziness, confusion, anxiety, agitation, tremors, weakness, hyperventilation, dyspnea, apnea, severe hypoxic signs in absence of cyanosis (cyanosis is generally late finding), convulsions, seizures, memory loss, insomnia, metabolic acidosis, poor appetite.

Chronic Potential Health Effects:
Skin: Prolonged or repeated skin contract may cause dermatitis.
Ingestion: Prolonged or repeated exposure from ingestion may affect the urinary system, brain, liver and thyroid (goiter) as well have the same effects as acute overexposure.

Section 12: Ecological Information

Ecotoxicity: Not available.

BOD5 and COD: Not available.

Products of Biodegradation:
Possibly hazardous short term degradation products are not likely. However, long term degradation products may arise.

Toxicity of the Products of Biodegradation: The products of degradation are less toxic than the product itself.

Special Remarks on the Products of Biodegradation: Not available.

Section 13: Disposal Considerations

Waste Disposal:
Waste must be disposed of in accordance with federal, state and local environmental control regulations.

Section 14: Transport Information

DOT Classification: CLASS 6.1: Poisonous material.

Identification: : Potassium cyanide UNNA: 1680 PG: I

Special Provisions for Transport: Marine Pollutant

Section 15: Other Regulatory Information

Federal and State Regulations:
Connecticut hazardous material survey.: Potassium cyanide
Illinois chemical safety act: Potassium cyanide
New York acutely hazardous substances: Potassium cyanide
Rhode Island RTK hazardous substances: Potassium cyanide
Pennsylvania RTK: Potassium cyanide
Minnesota: Potassium cyanide
Massachusetts RTK: Potassium cyanide
Massachusetts spill list: Potassium cyanide
New Jersey: Potassium cyanide

Other Regulations:
OSHA: Hazardous by definition of Hazard Communication Standard (29 CFR 1910.1200).
EINECS: This product is on the European Inventory of Existing Commercial Chemical Substances.

Other Classifications:

WHMIS (Canada):
CLASS D-1A: Material causing immediate and serious toxic effects (VERY TOXIC).
CLASS E: Corrosive solid.
WHMIS Class B-6: Reactive and very flammable material.

DSCL (EEC):
R16- Explosive when mixed with oxidizing substances.
R28- Very toxic if swallowed.
R38- Irritating to skin.
R40- Possible risks of irreversible effects.
R41- Risk of serious damage to eyes.
S1/2- Keep locked up and out of the reach of children.
S26- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28- After contact with skin, wash immediately with plenty of [***]
S36/37- Wear suitable protective clothing and gloves.
S39- Wear eye/face protection.
S45- In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
S46- If swallowed, seek medical advice immediately and show this container or label.

HMIS (U.S.A.):

Health Hazard: 3

Fire Hazard: 0

Reactivity: 0

Personal Protection: j

National Fire Protection Association (U.S.A.):

Health: 3

Flammability: 0

Reactivity: 0

Specific hazard:

Protective Equipment:
Gloves.
Synthetic apron.
Vapor and dust respirator. Be sure to use an approved/certified respirator or equivalent. Wear appropriate respirator when ventilation is inadequate.
Splash goggles.

Section 16: Other Information

References: Not available.

Other Special Considerations: Not available.

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QUALITY ASSURANCE:
GMP POPCORN EXERCISE

GMP POPCORN EXERCISE CHECKLIST

Goal:
Make a batch of GMP popcorn within a specified timeframe (1.5 hours).

Objective:
Understand the complexity of a GMP process.
Gain appreciation of teamwork and cooperation of all departments.

How it mirrors industry:

- You will be frustrated at times
- You will be rushed.
- You will feel a sense of accomplishment once you made the batch!
- The actual time for the chemistry / fermentation / etc. is very small compared to the time it takes to get all GMP documentation in place.

Supplies:

1. Department tasks and deliverables
2. Approved labels
3. Quarantine labels
4. Box for approved and quarantine
5. Microwave Popcorn
6. Access to photocopier
7. Access to microwave
8. Water in a squirt bottle
9. 409 or other type of cleaning agent
10. Paper towels
11. Sponge
12. Measuring cups
13. Bags for the finished product

Teams:
Material Control: 2 people
QC: 2 people that like to test material
QA: 2 people that have an eye for written details
Production: 4 people that like to WORK

Q U A L I T Y A S S U R A N C E

ROLE OF QA:

- Review and Approval all quality related documents.
- Issue all controlled documents.
- Provide oversight on the production campaign.
- Disposition Raw materials and final products.

Checklist of Items to Accomplish:

- Review and Approve Raw Material Master Specification Sheet
- Review and Approve Final product Master Specification Sheet
- Review and Approve Master Batch Record
- Approve actual Raw Materials for use
- Inspect Microwave for Cleanliness
- Review completed Batch Record after production is complete
- Review QC data and Approve actual Popcorn

Your Tasks are:

1. APPROVING MASTER SPECIFICATIONS:

Production will be submitting for your review AND approval:

1. **RAW MATERIAL SPECIFICATION SHEET** for the KERNELS
2. **FINAL PRODUCT SPECIFICATION SHEET** for the POPCORN.

Instructions: *Review to ensure all boxes that are **shaded** have been completed. All information provided should make sense and be reasonable. Try where possible to have the team write in quantitative specifications (i.e. "No more than 2 dark pieces of popcorn."). If you don't agree with the specifications, or have questions, feel free to go back to the Production Team for clarification.*

HOW TO APPROVE A MASTER SPECIFICATION SHEET:

To make the **specification sheet** effective complete the following:

a). **Sign** your name in the shaded box for "QA Approval".

b). **Write** in an **effective date** (i.e. today's date) in the upper right hand corner.

c). Make a **photocopy** (yes, go to the photocopier) of the original document you just signed and give a copy to QC.

d). **File** the original in QA.

2. APPROVING A MASTER BATCH RECORD:

Production will be submitting for your review:

1. A Master **BATCH RECORD** to make the POPCORN.

Instructions: *Review to ensure all boxes that are **shaded** have been*

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completed. All information provided should make sense and be reasonable. HINT: The production team should have quantitative numbers in the process (i.e. Pop popcorn for 1.5 – 2.5 minutes). If you don't agree with the process description or have questions feel free to go back to the Production Team for clarification.

HOW TO APPROVE A MASTER BATCH RECORD:

To make the BATCH RECORD effective complete the following:

a). **Sign** your name in the shaded box for "QA Approval" on the front page and initial the bottom of all pages in the QA box designated.

b). **Write in** an **effective date** (i.e. today's date) in the upper right hand corner on ALL PAGES.

c). Make a **photocopy** of the Master Batch Record

d). Write in the **Lot Number** on all pages (in the box for LOT NUMBER) **ON THE PHOTOCOPY OF THE BATCH RECORD**. (See below for instructions):

INSTRUCTIONS FOR ASSIGNING A UNIQUE LOT NUMBER:

Lot number should be POP-YEAR-001. For example: POP-03-001

e). **File** the original batch record in QA.

f). **Give the photocopy** of the batch record to production. (*We call this "Issuing a batch record to production*")

3. APPROVING RAW MATERIALS:

Before production can use the kernels in their production run they must be approved by QC AND QA! Follow the instructions below to approve the raw materials (i.e. Kernals).

HOW TO APPROVE RAW MATERIALS:

1. Once QC finishes the "testing" on the Kernals, **review** the information written in on the specification sheet and the accompanying documentation to ensure no empty spaces.
2. If acceptable, sign your name in the box for QA at the bottom of the page on the **specification sheet** and check off "**Approved**".
3. Fill in the spots on the **Approval labels. Make one for each container / package PLUS 1 extra for the receiving report.**
4. Complete the remaining boxes designated "QA" on the **Receiving report**. Place your extra label on the receiving report.
5. Give **approval labels** to Material Control.

4. INSPECTING EQUIPMENT:

Before production can use a piece of equipment it must be clean! It is a common practice in industry to have QA inspect the equipment after production cleans it.

HOW TO INSPECT EQUIPMENT:

1. Production will be requesting QA to **visually inspect** the microwave for cleanliness. If it is not satisfactorily clean, have Production re-clean the microwave.

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2. Once acceptable, sign/date the **Cleaning Log** in the spot for "QA initials"

5. INTERNAL AUDITING:

Feel free to audit the production area during production!

6. REVIEWING COMPLETED PRODUCTION BATCH RECORDS:

Once production of the popcorn is complete, the Production team will be submitting the completed batch record for your review. Before the popcorn is "Dispostioned" QA must review the completed batch record AND completed Quality Control testing. BOTH items must be satisfactorily before the popcorn can be approved!

HOW TO REVIEW A COMPLETED BATCH RECORD:

1. Ensure all information is recorded and completed per requirements of the batch record.
2. If any items were not completed –return to production for correction.
3. The team must not of deviated from requirements in the batch record. (i.e: if it says to "Pop the popcorn in the microwave for 2-3 minutes they must not go over 3 minutes or under 2 minutes without some justification.)
4. Once you are satisfied with the completed record, sign your name in the "Reviewed by QA" box
5. DO NOT YET APPROVE THE BATCH. YOU NEED THE QC DATA FIRST.

7. REVIEWING COMPLETED QC DATA

Once the QC data is complete – review the QC information for completeness. If both the Batch Record and QC data are acceptable you may sign both documents as "APPROVED". NOW THE POPCORN IS APPROVED.

HOW TO APPROVE QC DATA ON POPCORN

1. Once QC finishes the "testing" on the Popcorn, **review** the information written in on the specification sheet and the accompanying documentation to ensure no empty spaces.
2. If acceptable, sign your name in the box for QA at the bottom of the page on the **specification sheet** and check off "**Approved**".
3. If the batch record is complete and the QC data is complete you can now fill out the **Approval labels!**
4. Complete the remaining boxes designated "QA" on the **Receiving report**. **Make one label for each container / package PLUS 1 extra for the receiving report.**
5. Place your extra label on the receiving report.
6. Give **approval labels** to Material Control.

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M A T E R I A L C O N T R O L

ROLE OF MATERIAL CONTROL:

- MC controls the flow of materials and limits the access of materials to prevent against "off-grade" materials being used in production.
- Material Control inspects all incoming materials/packages for integrity.
- Material control offers expertise in packaging and storing of materials.

Your Tasks are:

1. **STORAGE AREAS**:

Designate two separate areas: "**Quarantine"** and "**Approved."** Use the boxes provided and label them appropriately.

2. **Inspect** all incoming packages for possible signs of damage during shipping.

3. **RECEIVING RAW MATERIALS:**

You will receive raw materials to enter into your system.

Instructions:

1. By using information on the package - fill in **SECTION 1** on the "**Receiving Report**". Use the template provide.
2. Give a **"RECEIVING NUMBER"** to the material. Use the following format: DDMMYY – 000
   (i.e. for the first item received on January 2, 2007 write: 010207-001)
3. Write this receiving number on the Receiving Report. Attach the COA to the Receiving Report.
4. Complete a **Quarantine Label** and place the completed **"Quarantine"** label on the material and transfer to the **"Quarantine" area**.
5. Give the "**Receiving Report**" and COA to QC- this is their cue to sample the material and begin testing.

4. **LABELING RAW MATERIALS APPROVED:**

1. QA will give Material Control "Approval" labels to apply to the material that is in "Quarantine".
2. Place the Approval Label to cover up the word "QUARANTINE" on the quarantine label. Move the material to the "Approved" area.
3. Production may now have the material. Give only "Approved" material to production for their use.

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5. RECEIVING FINAL PRODUCTS INTO QUARANTINE:

You will need to take the final product (i.e. popcorn) and place it in quarantine.

Instructions:

1. fill in **SECTION 1** on the "**Receiving Report**". Use the template provide. Ask production team for lot number.
2. Complete a **Quarantine Label** and place the completed **"Quarantine**" label on the material and transfer to the **"Quarantine" area**.
3. Give the "**Receiving Report**" to QC- this is their cue to sample the material and begin testing.
4. QA will give Material Control the "Approval" labels to apply to the material that is placed in "Quarantine". Move the material to the "Approved" area.

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P R O D U C T I O N

ROLE OF PRODUCTION:

- Execute the process according the batch record to produce a product within specifications
- Coordinate the batch record, release of raw materials, and equipment

Checklist of Items to Accomplish:

- ❑ Write Master Raw Material Specification Sheet and circulate for approvals
- ❑ Write Master Final Product Specification Sheet and circulate for approvals
- ❑ Write Master Batch Record and circulate for approvals
- ❑ Ensure Raw Materials have been tested and approved
- ❑ Clean Microwave per SOP
- ❑ MAKE GMP Popcorn! And complete batch record as you go!

HINT: PRODUCTION HAS MANY DOCUMENTS TO WRITE ….IT IS BEST IF YOU MULTI TASK AND SPREAD THE WORK AMONG THE DEPARTMENT.

Your Tasks are:

1. WRITING MASTER SPECIFICATIONS:

Production must write the following specifications and give to QC and QA to review and approve:

1. **RAW MATERIAL SPECIFICATION SHEET** for the KERNELS
2. **FINAL PRODUCT SPECIFICATION SHEET** for the POPCORN.

Instructions:

Use the template in your package. *Neatly complete all boxes (i.e. Vendor, storage conditions, specifications, etc.) that are* ***shaded*** *(except the signatures). All information provided should make sense and be reasonable. Try where possible to write quantitative specifications (i.e. "No more than 2 dark pieces of popcorn).*

1. Circulate for signatures –
   - 1st: Production signer in "Written By"
   - 2nd: Production Supervisor signs
   - 3rd: QC Supervisor
   - 4th: QA

NOTE: QA will keep the final document.

2. WRITING A MASTER BATCH RECORD:

Production must write a Master Batch Record for the production of Popcorn.
Use the Template provided.

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Instructions:
Enter all the information in the boxes that are ***shaded.*** *You must describe what you think your process will be. All information provided should make sense and be reasonable.*

HOW TO WRITE A MASTER BATCH RECORD:

a). Complete all shaded areas. Sign your name on the front page

b). Give to Production supervisor for review. Sign your name on the front page.

c). Give to QA to review. Sign your name on the front page.

d). Once QA is happy with the batch record, they will issue you a copy to conduct your production.

3. Don't forget to check with QC regarding the testing of your **raw materials (i.e.: Kernels)**.

4. CLEANING EQUIPMENT:

Before production can use a piece of equipment it must be clean! It is a common practice in industry to have QA inspect the equipment after production cleans it.

HOW TO CLEAN EQUIPMENT:

1. Use the SOP provided to clean your equipment (i.e. Microwave).
2. Once the production operator has cleaned the Equipment, complete the documentation required on the Cleaning log and have the Supervisor inspect the equipment.
3. Request QA to **visually inspect** the microwave for cleanliness.
4. Once QA has inspected the equipment and found it acceptable, you may now use the equipment.

5. STARTING PRODUCTION!

1. Once you have the issued batch record from QA; APPROVED raw material; and CLEAN equipment, **you may start production of the POPCORN.**
2. Follow the process in your batch record and document as you go. Once the production of the popcorn is complete so should your batch record.

ENDING PRODUCTION:

1. Notify Material Control to remove your popcorn from the equipment and place it in quarantine.
2. While QC is testing the material, the Production Operator must review the record to ensure all information is complete.

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3. Production supervisor must review and sign the back of the batch record.
4. Submit to QA for review.
5. Clean the equipment as documented above.
6. Wait to hear from QA if your material is approved!

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QUALITY CONTROL

ROLE OF QC:

- Test all materials to be used by Production.

Checklist of Items to Accomplish:

- Review Raw Material (i.e Kernals) Specification Sheet
- Test Raw Materials
- Review Final Product (i.e. Popcorn) Specification Sheet
- Test Final Product Materials

Your Tasks are:

1. APPROVING MASTER SPECIFICATIONS:

Production will be submitting for your review AND approval:

1. **RAW MATERIAL SPECIFICATION SHEET** for the KERNELS
2. **FINAL PRODUCT SPECIFICATION SHEET** for the POPCORN.

Instructions: *Review to ensure all boxes that are **shaded** have been completed. All information provided should make sense and be reasonable. Try where possible to have the team write in quantitative specifications (i.e. "No more than 2 dark pieces of popcorn). If you don't agree with the specifications or have questions feel free to go back to the Production Team for clarification.*

HOW TO APPROVE A MASTER SPECIFICATION SHEET:

To approve the **specification sheet** complete the following:

a). **Sign** your name in the shaded box for "QC Approval".
b). Give to QA for their review.

2. DOCUMENTS NEEDED:

NOTE: QA will give you copies of the specification sheets to document the results of the testing for BOTH the kernels and the popcorn.

3. APPROVING RAW MATERIALS:

Before production can use the kernels in their production run they must be approved by QC AND QA! Follow the instructions below to test and approve the raw materials (i.e. Kernals).

HOW TO TEST AND APPROVE RAW MATERIALS:

1. Material Control will be giving you a **RECEIVING REPORT** for both **KERNALS** and **POPCORN** (once made). This is your cue that something is in quarantine and needs to be tested by QC. Take your

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specification sheet to quarantine and begin to following the sampling and testing instructions.

2. Once the analyst completes the "testing" on the Kernals, **review** the information written in on the specification sheet and the accompanying documentation to ensure no empty spaces. The analyst will sign his/her initials and date in the column.
3. The QC Supervisor will review the package of data the analyst completed and If acceptable, sign your name in the box for QC approval at the bottom of the page on the **specification sheet** and check off "**Approved**".
4. Give package to QA.

4. **APPROVING FINAL PRODUCT (I.E: Popcorn)**:

Once production has made the popcorn, you will be required to test the material. Follow the instructions below to test and approve the final product (i.e. popcorn).

HOW TO TEST AND APPROVE FINAL PRODUCTS:

1. Material Control will be giving you a **RECEIVING REPORT** for the **POPCORN** (once made). This is your cue that something is in quarantine and needs to be tested by QC. Take your specification sheet to quarantine and begin to following the sampling and testing instructions.
2. Once the analyst completes the "testing" on the Popcorn, **review** the information written in on the specification sheet and the accompanying documentation to ensure no empty spaces. The analyst will sign his/her initials and date in the column.
3. The QC Supervisor will review the package of data the analyst completed and If acceptable, sign your name in the box for QC approval at the bottom of the page on the **specification sheet** and check off "**Approved**".
4. Give package to QA.

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Batch Production Record

Product Name: Popcorn

Effective Date:

Revision: 0

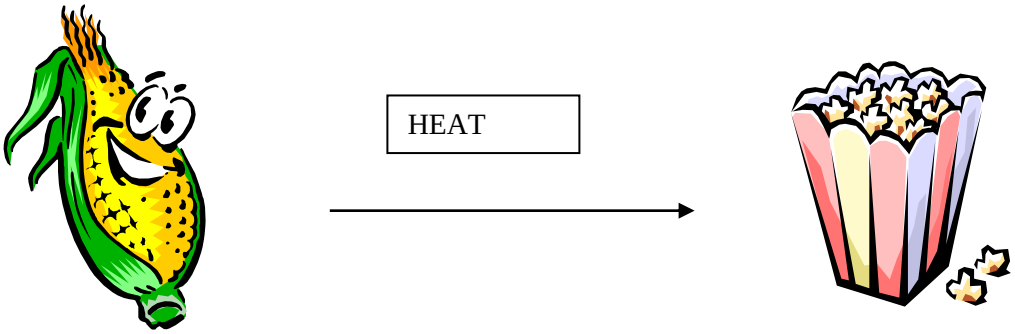
Written By:		**Date:**	
Production Supervisor Approval:		**Date:**	
Quality Assurance Approval:		**Date:**	

INPUT MATERIALS:

INPUT MATERIALS	PRODUCTION OPERATOR	CHECKED BY

Note:
Input Materials are materials used in your process (i.e. Kernals).

REACTION SCHEME



Document Approval

Quality Assurance Initial:	

EQUIPMENT CHECKLIST

(Standard batch size)

		Inventory Number	Production Operator Initial and date
	Microwave Oven		

Document Approval

Quality Assurance Initial:	

MANUFACTURING DIRECTIONS FOR LAB SCALE BATCH:

(completed by QA when issued)

Lot Number:

(Completed by QA on Master)

Effective Date:

Revision: 0

COMPOUND: POPCORN

PROCEDURE

Date:

		Operator init./time	Coworker init./time
1.			
2.			
3.			
4.			
5.			
6.			

Document Approval

Quality Assurance Initial:	

MANUFACTURING DIRECTIONS FOR LAB SCALE BATCH:

(completed by QA when issued)

Lot Number:

(Completed by QA on Master)

Effective Date:

Revision: 0

COMPOUND: POPCORN

Date:

		Operator init./time	Coworker init./time
7.			
8.			
9.			
10.			

Comments: (initial and date any comments)

Document Approval

Quality Assurance Initial:	

SUMMARY OF RESULTS

Actual Yield %:	%
Operator:	Supervisor:

STORAGE OF MATERIAL

Total containers of product transferred to storage:

STORAGE CONDITIONS: Store at room temperature.

CONCLUSION

Date Production Finished:	[ ]	Production Operator Initials:	[ ]
Production Supervisor Approval:	[ ]	Date:	[ ]
Quality Assurance Reviewer:	[ ]	Date:	[ ]

DISPOSITION OF LOT # _____.___.____.

Approved [ ] **Non-Conforming** [ ]

Quality Assurance Signature:		Date:

Document Approval

Quality Assurance Initial:	

RECEIVING REPORT

SECTION I : RECEIVING (Completed by Material Control)	
Material Name:	**Date Received:**
Supplier:	**Receiving Lot No.:**
Quarantine Label Applied: Yes ☐	**Number of Containers:**
Completed by:	**Date:**

SECTION II: SAMPLING and INSPECTION (Completed by QC)			
Total sample quantity (if final product otherwise N/A):	**Number of Containers Sampled / Inspected:**	**By:**	**Date:**

SECTION III: LABELING (Completed by QA)		
Number of labels issued: (QA)	**By**	**Date:**
Number of sample labels: (QA)	**By**	**Date:**
Attach Sample Label Below (QA)	**By:**	**Date**

Place sample label below:

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RAW MATERIAL SPECIFICATION SHEET

Receiving Number:		**Revision:**	**Effective Date:**

Item Description: KERNELS		
Structure: 		**Written By:** **Date:**
		QC Approval: **Date:**
		Production Supervisor Approval: **Date:**
		QA Approval: **Date:**
Hazards:	**MAY HARM TEETH!**	

Storage Condition:		**Supplier:**	

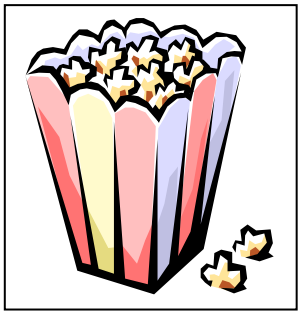
SPECIFICATIONS AND RESULTS

Test	Method	Specification	Result	QC Analyst Init. / Date
Physical Description	**Visual**			

QC Approval	❑ **Approved** ❑ **Non-Conforming**	**Date:**
QA Approval	❑ **Approved** ❑ **Non-Conforming**	**Date:**

FINAL PRODUCT SPECIFICATION SHEET

Lot Number:		Revision:	Effective Date:

Item Description: POPCORN		
Structure: 	Written By: Date:	
	QC Approval: Date:	
	Project Team Approval: Date:	
	QA Approval: Date:	
Hazards:		

Storage Condition:		Supplier:	

SPECIFICATIONS AND RESULTS

Test	Method	Specification	Result	QC Analyst Init./Date
Physical Description	Visual			

QC Approval	❑ Approved ❑ Non-Conforming	Date:
QA Approval	❑ Approved ❑ Non-Conforming	Date:

Receiving/Lot#:_________________
Initials.:_______ Date:_________
Storage Conditions:_____________
QUARANTINE

Receiving/Lot#:_________________
Initials.:_______ Date:_________
Storage Conditions:_____________
QUARANTINE

Receiving/Lot#:_________________
Initials.:_______ Date:_________
Storage Conditions:_____________
QUARANTINE

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Receiving/Lot#:_________________
Initials.:_______ Date:_________
Storage Conditions:_____________
QUARANTINE

Receiving/Lot#:_________________
Initials.:_______ Date:_________
Storage Conditions:_____________
QUARANTINE

STANDARD OPERATING PROCEDURE

Procedure:	Procedure No.: CLN-0001	Revision: 0
PROCEDURE FOR THE PRODUCT CHANGEOVER CLEANING OF A MICROWAVE OVEN	Effective Date: 01/15/07	
	Replaces Document: New	
Written by: Dept.: Production Date: 01/10/07	Dept. Approval: Dept.: Engineering Date: 01/10/07	
Content Review: Dept.: Engineering Date: 01/10/07	QA Approval: Date: 01/13/07	

I. PURPOSE

This procedure is to ensure proper cleaning of equipment.

III. PROCEDURE

		RESPONSIBILITY
3.1	**Product Changeover Cleaning**	
3.1.1	Enter the lot number of the batch on the "Equipment Cleaning Log".	Production
3.1.2	Clean the equipment using water as a cleaning agent and paper towels as cleaning implements.	Production
3.1.3	First wipe the interior top of the microwave from back to front.	Production
3.1.4	Wipe the interior back of the microwave from top to bottom.	Production
3.1.5	Wipe the interior sides of the microwave from back to front, then top to bottom.	Production
3.1.6	Wipe the interior bottom of the microwave from back to front. If the microwave has a turntable inside, remove pieces and clean them using water as a cleaning agent. Visually inspect for contaminants. Replace them in the microwave when complete.	Production
3.1.7	Complete the "Cleaning Log". Enter the SOP number used, cleaning agent, and time / date of person performing cleaning.	Production
3.1.8	Visually inspect for contaminants. If contaminants are present, repeat steps 3.1.2 through 3.1.7. If acceptance, initial column.	Supervisor
3.1.9	Notify QA to Inspect the equipment and verify that it is clean. Quality Assurance will sign the log in the "QA Initials Column" and check the product changeover box.	Production QA

EQUIPMENT CLEANING LOG

Equipment Name:_____Microwave_________________ Make.:______________

Lot Number of batch in production	Time/Date of Cleaning	1. SOP No. 2. Solvents Used	Initials of person performing cleaning and a coworker verifying	Cleaning Status
Lot Number: ____________________	**Time:** ______ **Date:** __________	SOP # _____________ Solvent: ______________	Production Initials: _____________ Supervisor Initials: _____________	❑ Product Changeover QA Initials:______ Date:______
Lot Number: ____________________	**Time:** ______ **Date:** __________	SOP # _____________ Solvent: ______________	Production Initials: _____________ Supervisor Initials: _____________	❑ Product Changeover QA Initials:______ Date:______
Lot Number: ____________________	**Time:** ______ **Date:** __________	SOP # _____________ Solvent: ______________	Production Initials: _____________ Supervisor Initials: _____________	❑ Product Changeover QA Initials:______ Date:______

QC MICROBIOLOGY

SOP: Operation of MetOne Model 229 B Laser Particle Counter

Approvals

Preparer: Sheila Byrne — Date: 23OCT06
Reviewer: Dr. Maggie Bryans — Date: 30JUN14

1. **Purpose:**
   1.1. Operation of the MetOne Laser Particle Counter.
2. **Scope:**
   2.1. Applies to the MetOne Laser Particle Counter for performing sampling of air quality.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. MetOne Model 228/229 Particle Counter Operating Guide
5. **Definitions: N/A**
6. **Precautions: N/A**
7. **Materials:**
   7.1. MetOne Unit, Model 229B
   7.2. Isokinetic Probe
   7.3. Purge Filter
   7.4. MetOne Battery Charger
8. **Procedure:**
   8.1. Operation
      8.1.1. Place the MetOne unit on the bench or cart at the appropriate location facing into the center of the room . Battery or AC power source may be used.
      8.1.2. Remove the red inlet cap from sensor inlet tube and attach the isokinetic probe.
      8.1.3. Turn on the MetOne unit using the switch on the side of the unit. After power on the model 229 will start counting and the display will be continuously updated as particles are detected. The counter will run for one minute then hold the displayed count until the unit is turned off. This count represents the number of particles in one tenth of a cubic foot of air. (To determine the number of particles in $1m^3$ of air divide by 0.002832.) Record this value.
      8.1.4. Turn off the MetOne unit.
      8.1.5. Remove isokinetic probe and replace red inlet cap on sensor inlet tube.
      8.1.5. Store unit.
   8.2. Recharging the MetOne battery.
      8.2.1. When the “low battery” display appears on the MetOne unit, the battery needs to be recharged.
         8.2.1.1. Turn off the MetOne unit and connect the AC adapter to the unit.
         8.2.1.2. Plug in the AC adapter and charge the battery for 16 hours.

SOP: Operation of MetOne Model 229 B Laser Particle Counter

8.3. Contamination.

8.3.1. When the counter is used in high concentration or uncontrolled environments, it is possible for the sensor to become contaminated. If this happens the sensor light will come on. If this occurs, replace the isokinetic probe with the purge filter.

8.3.2. Run the counter until the display reads 0 counts.

8.3.3. This usually clears the contamination and the sensor light will go out. (The sensor light also comes on if the laser diode fails.)

9. History

Revision Number	***Effective Date***	***Preparer***	***Description of Change***
0	23OCT06	Sheila Byrne	Initial release

SOP: Operation of Met One GT-321 Particle Counter

Approvals

Preparer: John Buford Date: 17OCT13
Reviewer: Tim Kull Date: 18OCT13
Reviewer: Dr. Maggie Bryans Date: 19OCT13

1. **Purpose**
   1.1. Measure the number of airborne particulates per cubic foot using the Met One GT-321 particle counter.
2. **Scope and Applicability**
   2.1. The Met One GT-321 particle counter measures airborne particles by drawing in ambient air using a vacuum pump and counting particles using a laser based diode sensor. This SOP covers normal operations including air sampling, battery recharging, and zero count testing.
3. **Summary of Method**
   3.1. Place the unit in the area to be measured.
   3.2. Attach the iso-kinetic probe.
   3.3. Turn on power and press START; 10 samples are taken automatically and averaged.
   3.4. Recharge the battery when "Low Battery" message is displayed.
   3.5. Periodically test the unit for air leaks using a zero count test.
4. **References**
   4.1. GT-321 Hand Held Particle Counter Operation Manual, GT-321-9800 Rev D.
5. **Definitions**

Iso-kinetic Constant movement or velocity

6. **Precautions**
   6.1. The GT-321 unit contains no user serviceable parts. Do not open or attempt to change the internal battery pack.
   6.2. Contains a Class I laser, considered not hazardous when properly operated. Repairs should be performed by manufacturer train service personnel only.
7. **Responsibilities**
   7.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   7.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

SOP: Operation of Met One GT-321 Particle Counter

8. Equipment and Materials

8.1. Met One GT-321 Particle Counter
8.2. Iso-kinetic probe
8.3. AC to DC converter module
8.4. Zero test filter

9. Procedure

9.1. ***Air sampling:***

9.1.1. Place the MetOne unit on a bench or cart in the area to be measured with the inlet nozzle pointing upward. Battery or AC power source may be used.
9.1.2. Remove the rubber cap from the inlet nozzle on the top of the unit and attach the iso-kinetic probe. (The iso-kinetic probe helps reduce errors due to the aerodynamic properties of small particles.)
9.1.3. Turn on the power switch on the left side of the case. The LCD display should indicate 0.3u.
9.1.4. (Optional) Press the SELECT key to cycle through the particle sizes: 0.3u, 0.5u, 1.0u, 2.0u, and 5.0u. Stop when 0.3u is displayed.
9.1.5. Press the START/STOP button on the front of the unit. The internal vacuum pump will start running. The unit will automatically take 10 ambient air samples, one cubic foot per sample. After 9 seconds the first reading will appear on the display representing the number of particles that are larger than the size selected above. Additional readings will appear every 6 seconds for a total of 10 samples.
9.1.6. When all 10 samples are taken, the average is computed and displayed. This count represents the number of particles in one cubic foot of air. Record this value. (To determine the number of particles in one cubic meter, divide by 0.02832.)
9.1.7. Turn off the MetOne unit.

9.2. ***Recharging the battery:***

9.2.1. When the battery needs recharging, the “Low Battery” message is displayed and the pump will not activate when the START button is pressed.
9.2.2. Turn off the unit and connect the AC to DC converter module.
9.2.3. Plug in the AC to DC converter module and charge the battery for 15 hours.

9.3. ***Zero count test:***

9.3.1. Periodically test the Met One unit for air leaks. (Weekly is recommended.)
9.3.2. Remove the rubber cap from the inlet nozzle on the top of the unit and attach the zero test filter.
9.3.3. Turn on the power switch and press the START/STOP button as if air sampling.
9.3.4. The result of the 1 minute sampling should be zero. If it does not read zero, the unit may need to be repaired by the factory.

SOP: Operation of Met One GT-321 Particle Counter

10. Attachments

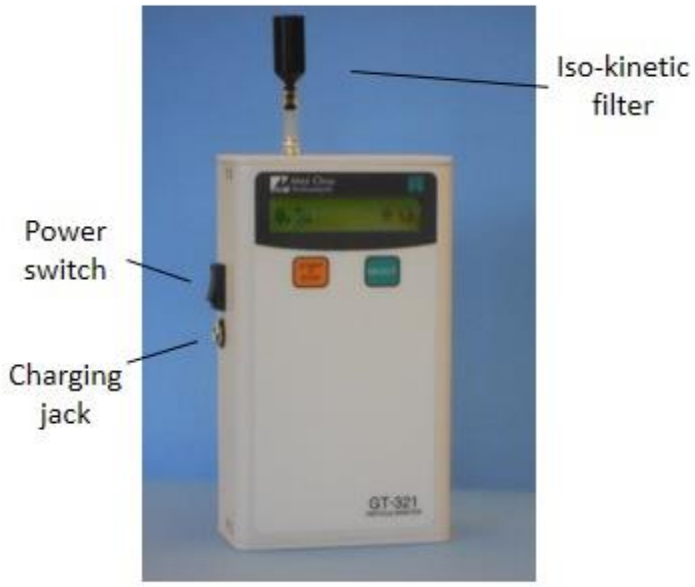


Figure 1. Met One GT-321 Particle Counter

11. History

Revision Number	***Effective Date***	***Preparer***	***Description of Change***
0	10/17/2013	John Buford	Initial release

SOP: M Air T Millipore Air Tester

Approvals:

Preparer: Dr. Maggie Bryans — Date: 09JAN14
Reviewer: Jason McMillan — Date: 10JAN14

1. **Purpose:**
   1.1. The purpose of this SOP is to describe the procedure in using the M Air T Millipore Air Tester in conducting airborne microbial testing.
2. **Scope:**
   2.1. The scope of this SOP is limited to performing airborne microbial testing using the M Air T Millipore Air Tester.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor /lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. M Air T Millipore Air Tester Operation and Maintenance Instruction
   4.2. autoclave SOP
   4.3. incubator SOP
5. **Definitions:** N/A
6. **Precautions:**
   6.1. Always wear the appropriate personnel protective equipment (safety eye glasses and gloves).
7. **Materials:**
   7.1. M Air T Millipore Air Tester and accessories.
   7.2. M Air T Cassette pre-filled with TSA media
   7.3. autoclave
   7.4. incubator
   7.5. 70% isopropyl alcohol (IPA)
   7.6. lab towels
8. **Procedure:**
   8.1. **Using the air tester in vertical, horizontal or inclined position**
      8.1.1. When using the air tester in a vertical position, the tripod is not used.
      8.1.2. When using the air tester in a horizontal position or 30° from the horizontal position, the tripod is needed. Fix the tripod onto the air tester by screwing it into the tester fixing hole.
   8.2. **Powering up the equipment**
      8.2.1. If the equipment is used to collect samples inaccessible to power outlets, the equipment has internal rechargeable batteries ready for use. Press the ON/OFF button. LCD display will be turned on.
      Note: Make sure the battery is fully charged. The LCD will display the battery symbol if it is fully charged.

SOP: M Air T Millipore Air Tester

8.2.2. If the equipment is used to collect samples accessible to power outlets, plug the power adapter into an empty and convenient power outlet. LCD display will be turned on.

8.3. **Adjusting the volume to be processed**

Note: Refer to Figure 2.

8.3.1. The recommended volume is 1000 Liters.

8.3.2. Setting up volume other than the recommended volume

8.3.2.1. To access other preset volumes, press the LITERS button multiple times until you find your desired volume.

8.3.2.2. To change the volume setting, select the volume that is just below the preset volume you want to process. Then hold the LITERS button until the tester display indicates the desired sampling volume.

8.4. **Adjusting the timer**

Note: Refer to Figure 2.

8.4.1 The recommended set time is 5 minutes.

8.4.2 Setting up the time other than the recommended set time.

8.4.2.1 Hold down the START/DELAY button. The display shows preset times in increments of 5 minutes up to one hour. Select the desired time by simply releasing the START/DELAY button.

8.5 **Installing the cassette and running the tester**

Note: Refer to Figures 2 and 3 as needed.

8.5.1 Define the location of the tester according to cGMP requirements

8.5.2 Ensure that pre-filled cassettes with TSA media are at room temperature before starting the test.

8.5.3 Spray down gloved hands with 70% IPA.

8.5.4 Sanitize the external surfaces of the tester with 70% IPA.

8.5.5 Position the wings of the cassette into the recessed area of the tester head.

8.5.6 Retain the cassette in position by holding on to its wings. Remove the lid and place it on the bench, internal face down.

8.5.7 Lock the micro-perforated sieve into position and remove cover

8.5.8 Press the ON/OFF button. Set the default volume and time, and then quickly press the START/DELAY button twice.
Note: The first 500 liters of volume of air collected is a slow flow rate producing a mild sound while the remaining 500 liters is a faster flow rate producing a noisy sound.

8.5.9 When the display indicates end of cycle (EOC) which is related to the set time, unlock the sieve, remove it, and put the lid back on the cassette.

8.5.10 To remove the cassette from the tester head, lift the cassette while firmly holding the edge.

8.5.11 Label (includes relevant sample data, date, initial and testing location) and incubate the cassette (37ºC for 1-3 days) in the upside down position.

8.5.12 Remove the cover from the sieve. Autoclave the sieve (without the cover) for 30 minute at 121°C.

8.5.13 Sanitize the external surfaces of the tester with alcohol.

SOP: M Air T Millipore Air Tester

8.5.14 Record use in the usage log.

8.6 **Evaluation of results.**

8.6.1 When incubation is complete, count the colonies on the plate.

8.6.1.1 The microbial count (CFUs) is to be stated with reference to the sample volume (i.e. CFUs/Sample Volume)

8.6.1.2 Record results in the log book.

9. Attachments:

9.1. Figure 1: Diagram of the M Air T Millipore Air Tester with Components
9.2. Figure 2: Diagram of the M Air T Millipore Air Tester with Display Buttons
9.3. Figure 3: Diagram of the M Air T Millipore Air Tester with Installed Cassette
9.4. Table 1: Environmental Monitoring Testing Sheet

10. History:

Name	Date	Amendment
Marlo Austria	01APR06	Initial Release
Bob O'Brien	11SEP06	Updated procedure. Changed usage log. Added Environmental Monitoring Testing Sheet.
Bob O'Brien	14MAR07	Updated date format. Updated Figures 3 and 4.
Jason McMillan	10JAN14	Changed College Name, Added Testing Sheet

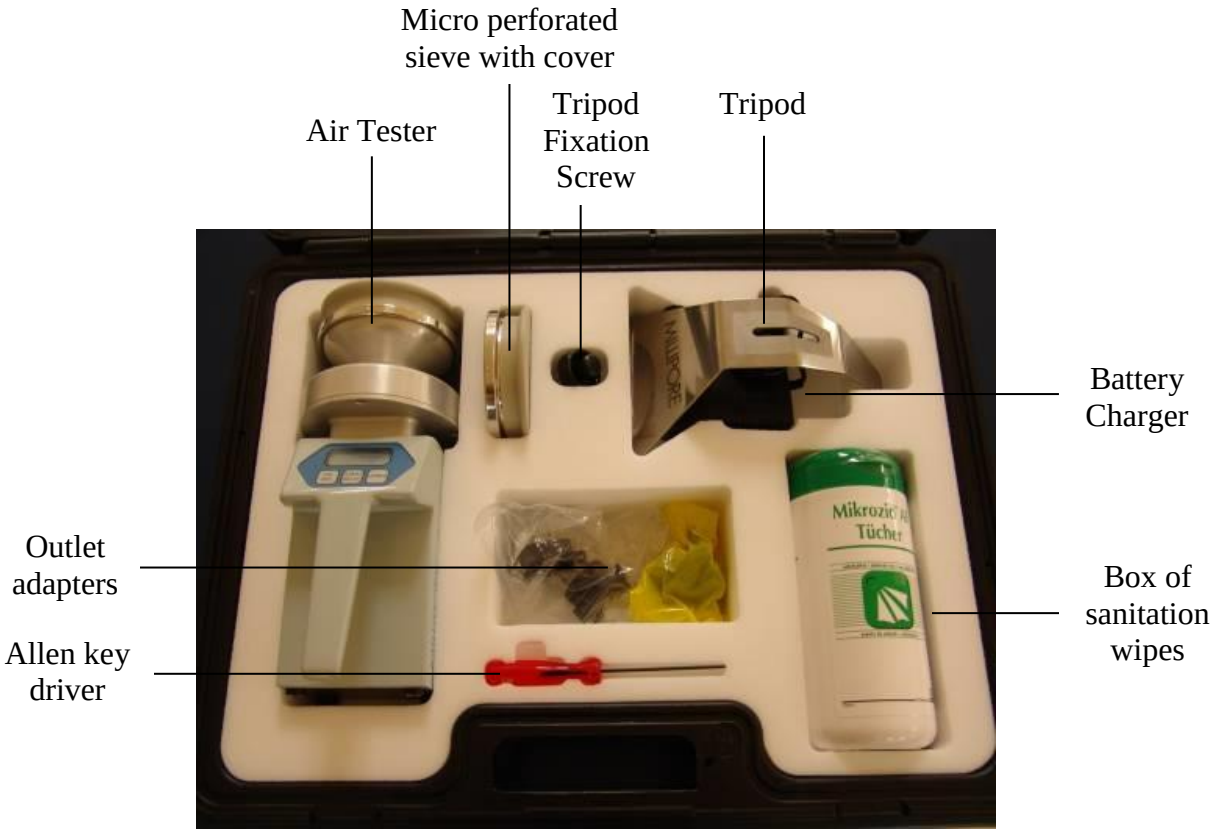


Figure 1: Diagram of the M Air T Millipore Air Tester with Components

SOP: M Air T Millipore Air Tester

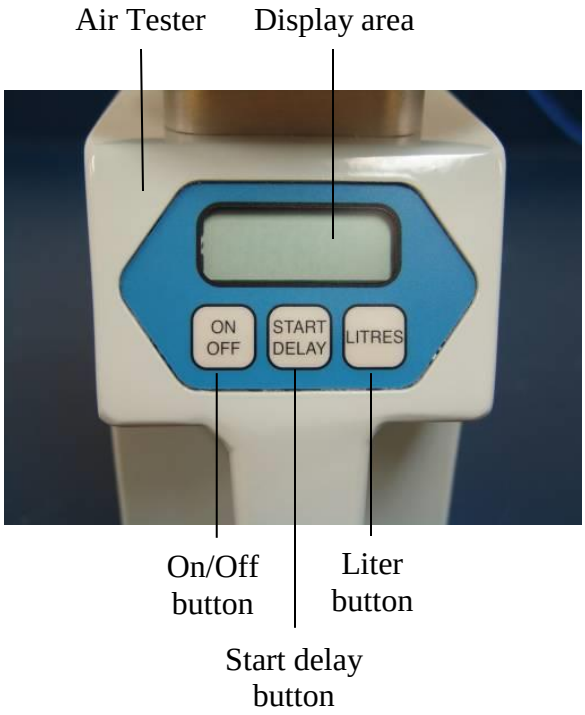


Figure 2: Diagram of the M Air T Millipore Air Tester with Display Buttons

Figure 3: Diagram of the M Air T Millipore Air Tester with Installed Cassette, Micro Perforated Sieve and Cap

SOP: M Air T Millipore Air Tester

Table 1: Environmental Monitoring Testing Sheet

Date Tested	Location	Air Volume (L)	Operator's Initials and Date	Number of Colonies	Plate Incubation Time	Pass or Pail (P/F)	Operator's Initials and Date

SOP: Gowning for Entry into Biomanufacturing Suite

Approvals:

Preparer: Dr. Maggie Bryans Date: 18FEB14
Reviewer: Jason McMillan Date: 19FEB14

1. **Purpose:**
   1.1. To describe the proper gowning procedure for personnel entering the biomanufacturing suite to minimize the number of particles and viable microorganisms in the suite.
2. **Scope:**
   2.1. Applies to gowning performed in the gowning area prior to entering the biomanufacturing suite.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Gowning for Aseptic Filling, Doxpub, Inc., Document number 02-0028-SOP-1.0
5. **Definitions:** N/A
6. **Precautions:**
   6.1. 70% isopropyl alcohol is flammable and poisonous if ingested. Avoid creating excessive mist when using spray bottles with IPA.
7. **Materials:**
   7.1. disinfecting hand soap
   7.2. sterile 70% (v/v) isopropyl alcohol (IPA)
   7.3. head cover
   7.4. hood (if needed)
   7.5. facial hair cover (if needed)
   7.6. cleanroom coverall, sterile Tyvek
   7.7. shoe covers
   7.8. non-powdered nitrile gloves
   7.9. lab tissues such as Kimwipes
8. **Procedure:**
   8.1. Employees should wear clean clothes that are not overly capable of shedding particulates (i.e., wool sweaters).
   8.2. Gowning must occur only when no one is entering or exiting the gowning area. Likewise, do not enter the gowning area while someone is gowning.
   8.3. Wearing makeup and jewelry is prohibited in the cleanrooms. If necessary, remove makeup and jewelry before proceeding to the Pre-Gowning Area.
   8.4. **Pre-Gowning Area**
      8.4.1. Enter the pre-gowning area.
      8.4.2. Sanitize hands with hand sanitizer.
      8.4.3. Sanitize safety glasses with 70% (v/v) IPA, dry with a lab tissue, and place on face.
      8.4.4. Don head cover. Use the mirror to verify that all hair is completely covered up to and including the hairline.

SOP: Gowning for Entry into Biomanufacturing Suite

8.4.5. If applicable, don beard cover and place over facial hair. Use the mirror to verify that all hair is completely covered.
8.4.5.1. If facial hair is still exposed, replace the head cover with a hood. Verify That all hair is completely covered.
8.4.6. Disinfect hands.
8.4.7. Proceed to gowning area.

8.5. **Gowning Area**

Note: Avoid creating excess mist while using IPA throughout this procedure.

8.5.1. Put on gloves. Choose gloves that have a snug but not overly tight fit.
8.5.2. Sanitize gowning bench with sterile 70% (v/v) IPA.
8.5.3. Sanitize gloves with sterile 70% (v/v) IPA.
8.5.4. Donning the shoe covers and coverall
8.5.4.1. Obtain a gowning package.
8.5.4.1.1. Inspect the integrity of the package and verify that the package has been autoclaved.
8.5.4.2. Open the package.
8.5.4.3. Inspect the shoe covers for rips or tears.
8.5.4.3.1. If rips or tears are present, discard the shoe covers in the receptacle and repeat step 8.5.5.1.
8.5.4.4. Sit on bench and put on a single shoe cover.
8.5.4.5. Swing leg over bench to clean side of gowning area. Apply other shoe cover and stand on clean side of gowning area.
8.5.4.6. Sanitize gloves with sterile 70% (v/v) IPA.
8.5.4.7. Remove the coverall from the package.
Note: Avoid touching the coverall to the floor at all times.
8.5.4.8. Inspect the coverall for rips or tears.
8.5.4.8.1. If rips or tears are present, discard the coverall in the waste receptacle and repeat step 8.5.4.1.
8.5.4.9. Unzip the coverall.
8.5.4.10. Gather the arms and one leg of the coverall together.
8.5.4.11. Place leg in coverall and pull up the coverall. Repeat the process with the other leg. Do not allow the sleeves to touch the floor.
8.5.4.12. Pull the coverall up over the body and zip.

8.6. Sanitize gloves with sterile 70% (v/v) IPA.
8.7. After gowning, employees may enter the biomanufacturing area. While performing aseptic processing, resanitize gloves as needed. Reglove or regown if any adverse circumstances are observed or suspected that may affect the integrity of the gown components.
8.7.1. Regown by exiting the biomanufacturing suite and repeating this SOP.

8.8. **Exiting the biomanufacturing suite**

8.8.1. Proceed to the exit area.
8.8.2. Discard coverall and other cleanroom garb appropriately in the receptacle.
8.8.3. Regown with fresh supplies when reentering the area.

8.9. Gowning qualifications for non-qualified personnel.

SOP: Gowning for Entry into Biomanufacturing Suite

8.9.1. Non-qualified personnel (employees, outside contractors, or visitors) may not enter the biomanufacturing suite without permission from the Director of Manufactruing or QA/QC.

8.9.2. Upon permission, non-qualified personnel must gown with instructions and in the presence of a fully qualified operator and be monitored using applicable standard procedures.

8.9.3. All occurrences of non-qualified personnel entering the biomanufacturing suite between the time of room sanitization and completion of a batch must be documented using a discrepancy report.

9. Attachments: N/A

10. History:

Name	Date	Amendment
Deb Audino	22OCT05	Initial Release
Deb Audino	06FEB05	Changed donning gown from dirty side to clean side.
Deb Audino	17JUL08	College name change
Jason McMillan	19FEB14	College name change

SOP: Gram Stain

Approvals:

Preparer: Dr. Maggie Bryans Date: 18FEB14
Reviewer: Jason McMillan Date: 19FEB14

1. Purpose:

1.1. To Gram stain samples.

2. Scope:

2.1. Applies to Gram staining samples using the 3-step method to detect the presence of Gram positive and Gram negative microorganisms.

3. Responsibilities:

3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.

3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

4. References:

4.1 Gram stain pack insert

4.2. microscope SOP

5. Definitions:

5.1. Gram positive microorganism: a microorganism that stains dark purple when treated with Gram staining solutions.

5.2. Gram negative microorganism: a microorganism that stains pink when treated with Gram staining solutions.

6. Precautions:

6.1. Gram Stain reagents are harmful. Wear gloves while performing this SOP.

7. Materials:

7.1. 4-step Gram stain kit
7.2. microscope slide
7.3. P20 pipet and tips
7.4. Bunsen burner
7.5. safety gas lighter with flint
7.6. tongs
7.7. inoculation loop
7.8. isopropanol
7.9. slide staining rack
7.10. timer
7.11. water
7.12. immersion oil
7.13. microscope with 1000X magnification
7.14. lab tissues
7.15. lab towels

8. Process:

Note: Refer to Figures 1-6 as needed before performing this SOP and throughout the procedure as needed.

8.1. **Sample preparation**

SOP: Gram Stain

8.1.1. Label a glass microscope slide with pertinent information.
8.1.2. Prepare slide following directions for the appropriate sample source:
8.1.2.1. If sample is from a liquid culture, pipet 10µL of the culture onto the microscope slide.
8.1.2.1.1. Spread into a thin film with the pipet tip.
8.1.2.2. If sample is from a colony, pipet 10µL of water onto the slide.
8.1.2.2.1. Take a sample of the colony using a sterile loop.
8.1.2.2.2. Place the loop full of sample on the glass microscope slide, mix with water and spread into a thin film.
8.1.3. Gently heat fix the microbes to the slide.
Note: Do not overheat the slide. Excessive heating will cause atypical staining.

8.2. **Gram stain**
8.2.1. Place the slide on a slide rack to cool to room temperature before staining.
8.2.2. Cover the fixed sample on the slide with crystal violet stain and leave for approximately 1 minute.
8.2.3. Wash with a stream of water until the water runs clear.
8.2.4. Cover the fixed sample on the slide with iodine mordant and leave for approximately 1 minute.
8.2.5. Wash with a stream of water until the water runs clear.
8.2.6. Rinse with decolorizer.
8.2.7. Wash with a stream of water until the water runs clear.
8.2.8. Cover the fixed sample with safranin and leave for 30-60 seconds.
8.2.9. Wash with a stream of cold water until the water runs clear.
8.2.10. Air-dry or blot with lab tissue.
Note: Do not rub glass slide with the lab tissue.
8.2.11. View with the light microscope at 100x magnification (using oil).
8.2.12. Record whether cells are Gram positive (dark purple) or Gram negative (pink).
8.2.13. Discard the slide in the biohazard sharps container.

9. Attachments: N/A
9.1. Figure 1: Taking sample colony
9.2. Figure 2: Spreading sample colony thin film
9.3. Figure 3: Heat fix sample
9.4. Figure 4: Sample covered with crystal violet
9.5. Figure 5: Sample covered with iodine mordant
9.6. Figure 6: Sample covered with safranin

10. History:

Name	Date	Amendment
Bob O'Brien	12Jun07	Initial release
Deb Audino	04Apr08	College name change
Jason McMillan	19FEB14	College name change

SOP: Gram Stain

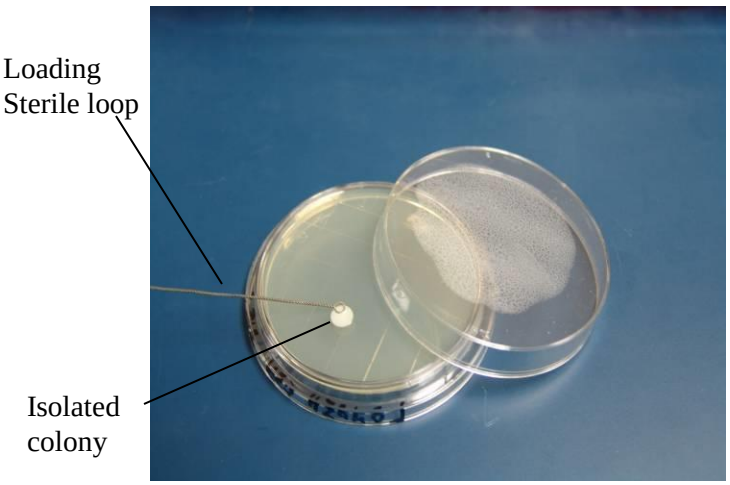


Figure 1: Taking sample colony

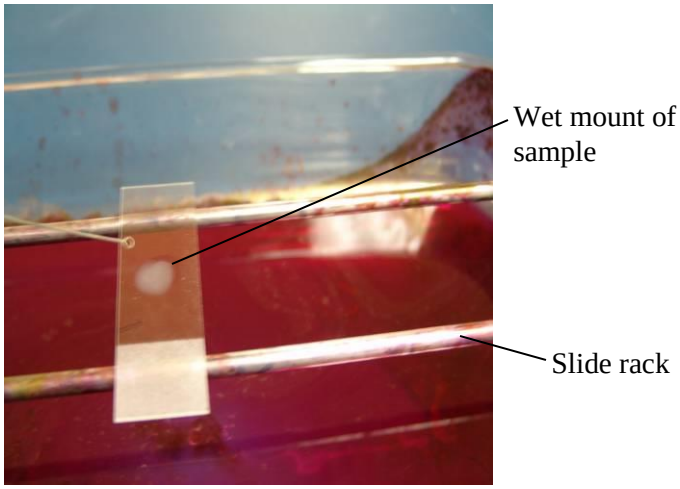


Figure 2: Spreading sample colony thin film

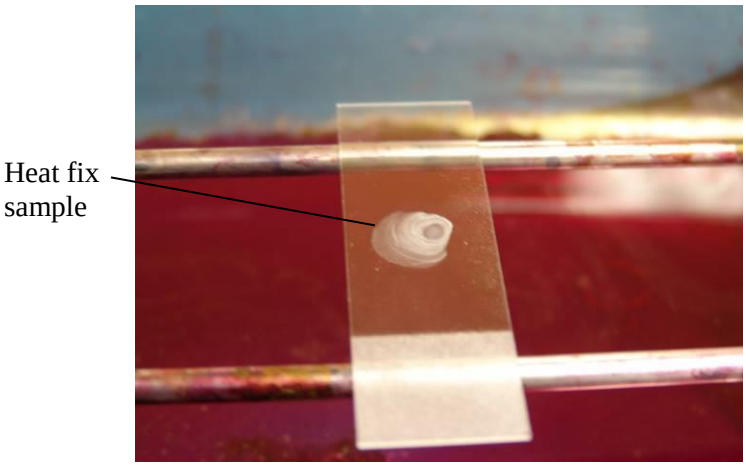


Figure 3: Heat fix sample

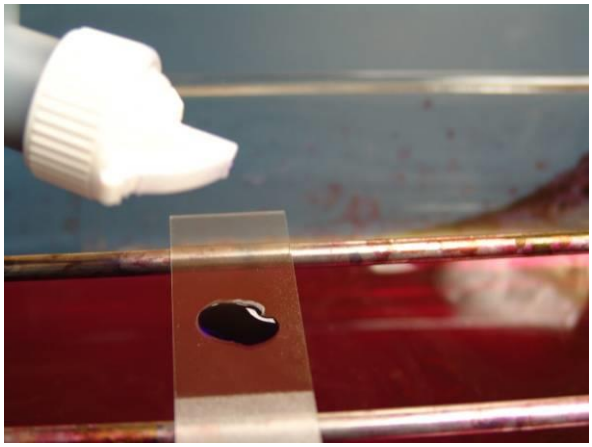


Figure 4: Sample covered with crystal violet

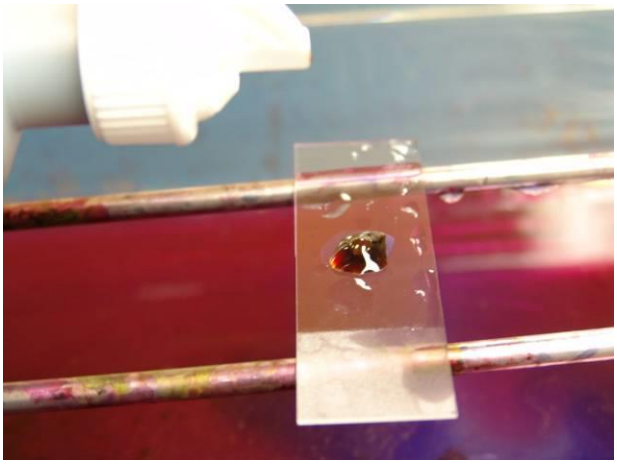


Figure 5: Sample covered with iodine mordant

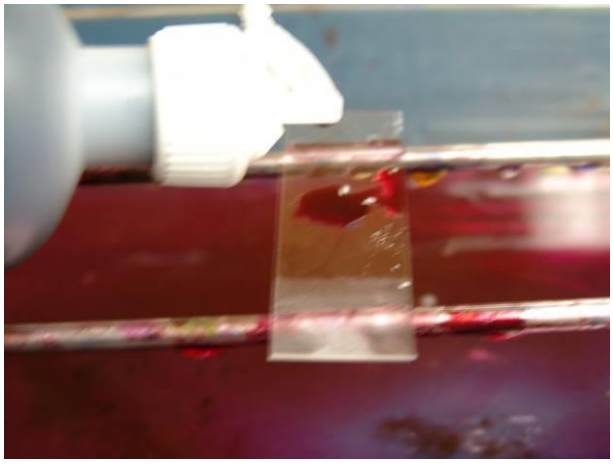


Figure 6: Sample Covered with safranin

SOP: LAL ASSAY - Gel Clot Method

Approvals:

Preparer: Dr. Maggie Bryans — Date: 09JAN14
Reviewer: Jason McMillan — Date: 10JAN14

1. **Purpose:**
   1.1. To perform the LAL Gel Clot Assay
2. **Scope:**
   2.1. To perform the LAL Gel Clot assay on various samples such as raw materials, in process materials and the final product for determination of endotoxin concentration.
3. **Responsibility:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. LAL pack instructions
   4.2. water bath SOP
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. LRW (LAL reagent water)
   7.2. LAL with a label sensitivity of 0.06EU/mL or 0.03EU/mL
   7.3. 5-10mL syringe and needle
   7.4. de-pyrogenated soda lime test tubes
   7.5. 100µl micropipette and sterile pipet tips
   7.6. laboratory film, such as Parafilm
   7.7. test tube rack
   7.8. 37°C water bath
8. **Procedure:**
   8.1. **Prepare the LAL Reagent**
      8.1.1. Reconstitute the LAL by adding LAL grade reagent water (LRW). Swirl occasionally until completely dissolved (about 3 minutes).
   8.2. **Dilute the Sample**
      8.2.1. Set up a row of 7 de-pyrogenated test tubes and label the tubes as: Undiluted, 1:2, 1:4, 1:8, 1:16, 1:32, Negative Control.
      Note: Keep tubes covered with laboratory film when not in use.
      8.2.2. Add 100µL LRW to all tubes EXCEPT the "Undiluted" tube using the same pipet tip.
      8.2.3. Add 200µL of the sample to the "Undiluted" tube. Change pipet tip.
      8.2.4. Tip the tube so that the liquid reaches the lip of the tube and remove 100µL of the liquid. Add it to the 1:2 tube.
      8.2.5. Vortex the tube for 4 seconds. Change pipet tip.

SOP: LAL ASSAY - Gel Clot Method

8.2.6. Tip the tube so that the liquid reaches the lip of the tube and remove 100µL of the liquid. Add it to the 1:4 tube.
8.2.7. Vortex mix the 1:4 tube for 4 seconds. Change pipet tip.
8.2.8. Tip the tube so that the liquid reaches the lip of the tube and remove 100µL of the liquid. Add it to the 1:8 tube.
8.2.9. Vortex mix the 1:8 tube for 4 seconds. Change pipet tip.
8.2.10. Tip the tube so that the liquid reaches the lip of the tube and remove 100µL of the liquid. Add it to the 1:16 tube.
8.2.11. Vortex mix the 1:16 tube for 4 seconds. Change pipet tip.
8.2.12. Tip the tube so that the liquid reaches the lip of the tube and remove 100µL of the liquid. Add it to the 1:32 tube.
8.2.13. Vortex mix the 1:32 tube for 4 seconds. Change pipet tip.
8.2.14. Tip the 1:32 tube, remove 100µL and DISCARD it.
8.2.15. Do not add sample to the negative control tube.
Note: All the tubes should have 100µL of liquid.

8.3. **Add the LAL Reagent**

8.3.1. Starting with the negative controls and proceeding from the lowest to the highest sample concentration, add 100µL LAL to each tube. Tips need to be changed after each addition.
Note: LAL must be added to all tubes within 2 minutes.
8.3.2. Shake the test tube rack vigorously for 30 seconds to mix the LAL and sample.

8.4. **Incubate the Tubes**

8.4.1. Cover the tubes with laboratory film and CAREFULLY place the rack in the water bath at about 37°C (Do not disturb other racks). Record the temperature and time.
Note: Do not disturb the tubes during the incubation. Once a clot is broken, it will not re-form.
8.4.2. Incubate for approximately 60 minutes.

8.5. **Analyze the Tubes**

8.5.1. Remove the tubes one at a time from the incubator and invert them SLOWLY and SMOOTHLY. Score tubes as positive if a firm clot has formed. Score tubes as negative if a gel holds, but collapses after the tube is fully inverted.
8.5.2. Record data.
8.5.3. Determine the amount of endotoxin in the samples using the formula:
Endotoxin concentration < LAL label sensitivity x dilution factor of most concentrated sample NOT to clot.

9. Attachments:

9.1. Data Table

SOP: LAL ASSAY - Gel Clot Method

10. History:

Name	Date	Amendment
Deb Audino	2001	Initial Release
Deb Audino	2003	Added more detailed directions.
Deb Audino	02Feb05	Replaced CSE with a sample.
Deb Audino	10Oct05	Added undiluted sample, data table, and how to calculate endotoxin level.
Deb Audino	04Apr08	College name change
Jason McMillan	10JAN14	College name change

	Undiluted 1	1:2	1:4	1:8	1:16	1:32	Negative Control
Sample ID _____							
Sample ID _____							

SOP: API 20E Microbial Identification

Approvals:

Preparer: Dr. Maggie Bryans Date: 17MAR14
Reviewer: Jason McMillan Date: 18MAR14

1. **Purpose:**
   1.1. To perform a microbial identification assay.
2. **Scope:**
   2.1. This procedure is intended as a standardized identification system for *Enterobacteriaceae* and non-fastidious Gram-negative rods included in the database.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. API 20E System Brochure
   4.2. Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing – cGMP (FDA publication, September 2004)
   4.3. United States Pharmacopeia 25
   4.4. Gram Stain SOP
   4.5. Bergey's Manual of Systematic Bacteriology
5. **Definitions:**
   5.1. *Enterobacteriaceae*: Family of Gram-negative, rod bacteria that inhabit soil, water and are commonly found in the large bowel of humans. Most common organisms isolated from clinical specimens.
6. **Precautions**: Aseptic technique and standard precautions for handling microbial cultures.
7. **Materials:**
   7.1. API 20E System strip, incubator tray and cover
   7.2. API 20E Results sheet
   7.3. API 20E Quick Index Booklet or the API 20E Profile Recognition System
   7.4. Test tube rack
   7.5. Disposable gloves
   7.6. Bacterial cultures including E. coli control (ATCC #25922)
   7.7. 10% ferric chloride
   7.8. 3% hydrogen peroxide
   7.10 McFarland No. 0.5 standard
   7.11. Nitrate reagents (I&II)
   7.12. Oxidase
   7.13. Zn dust
   7.14. Kovac's reagent
   7.15. VPI and VP2 reagents
   7.16. Sterile mineral oil
   7.17. Bacterial incubator set at 36°C

SOP: API 20E Microbial Identification

7.18. Test-tube rack

8. Procedure:

8.1. Using the Gram-stain technique (Gram-stain SOP) determine that the bacterial culture is a Gram-negative rod.

8.2. **Oxidase test**:

8.2.1. Using aseptic technique select an isolated colony from the streak plate.

8.2.2. Smear a small amount of the colony over a small area of filter paper.

8.2.3. Perform the oxidase test according to the manufacturer's instructions. Record the result on the API 20E Results sheet (21st identification test).

8.3. **Preparation of the inoculum**:

8.3.1. Open an ampule of API Suspension Medium (5 ml).

8.3.2. Remove a well-isolated colony from the streak plate and transfer to the API Suspension Medium. Mix to emulsify and obtain a homogenous suspension.

8.3.3. Check the turbidity of the suspension to that of the McFarland No. 0.5 standard. If necessary, add more bacteria.

8.3.4. The suspension must be used promptly after preparation.

8.4. **Preparation of the API strip**:

8.4.1. Obtain an incubation box (tray and lid).

8.4.2. Add 5 ml of distilled water to the bottom of the incubation tray.

8.4.3. Record your name, date and strain reference on the elongated flap of the incubation tray.

8.4.4. Remove the strip from the sealed pouch and place it in the incubation tray.

8.5. **Inoculation of the API strip**:

8.5.1. Gently shake the 5 ml of bacterial suspension.

8.5.2. Remove the cap and fill the 5-ml Pasteur pipette with the bacterial suspension.

8.5.3. Tilt the incubation strip to avoid forming air bubbles.

8.5.4. Using the Pasteur pipette, fill both the tube and the cupule of the tests CIT, VP and GEL.

8.5.5. Fill only the tube (NOT the cupule) of the other tests.

8.5.6. Overlay mineral oil and completely fill the cupule section of the tests ADH, LDC, ODC, H_2S and URE.

8.5.7. Place the lid on the incubation tray and incubate at 36°C ± 2°C for 18-24 hours.

8.5.8. Make an isolation streak on a TSA plate with a portion of the bacterial suspension to ascertain the purity of the sample.

8.6. **Reading the API strip**:

8.6.1. After the incubation period, read the strip by referring to the Reading Table.

8.6.2. If **3 or more tests** are positive, record all reactions not requiring the addition of reagents (do NOT read TDA, VP, IND). Record the results by placing a (+) for a positive reaction and a (–) for a negative reaction and continue with step 8.6.3.If the number of positive tests is **less than 3** reincubate the strip for a further 24 hours (± 2 hours) before continuing to step 8.6.3

8.6.3. Reveal the tests which require the addition of reagents and add the following test reagents in the order listed. In all cases, read the results immediately after adding

SOP: API 20E Microbial Identification

the reagents and waiting the proper length of time. Do not replace the lid on the tray until all results have been collected. Record results.

8.6.3.1. **TDA test**: Add 1 drop of 10% ferric chloride to the TDA tube. A reddish brown color indicates a positive reaction. A negative reaction is yellow.

8.6.3.2. **VP test**: Add 1 drop each of VP1 (40% KOH) and VP2 (α-napthol) solutions to the VP microtube. The KOH solution should be added first. Wait at least 10 minutes. A pink color developed in the whole cupule indicates a positive reaction. No color change is a negative reaction.

8.6.3.3. **IND test**: This test must be performed last. Add 1 drop of Kovac's/James reagent to the IND tube. A positive test is indicated by a red ring with 2 minutes. A yellow ring is a negative reaction.

8.7. Interpretation:

8.7.1. Identification is determined by the numerical profile.

8.7.2. On the result sheet, the tests are separated into groups of 3 and a value of 1, 2 or 4 is indicated for each. By adding together the values corresponding to positive reactions within each group, a 7-digit profile number is obtained for the 20 tests of the API 20 E strip. The oxidase reaction constitutes the 21st test and has a value of 4 if it is positive.

8.7.3. Identify the microorganism by entering the 7-digit numerical profile manually using the APIweb software.

8.7.4. Print out and record your results.

8.7.5. If the 7-digit profile is not discriminatory enough perform the following supplementary tests.

8.7.5.1. Reduction of nitrates to nitrites (NO_2) and N_2 gas: Add 1 drop each of NIT 1 and NIT2 to the GLU tube. Wait 2 to 5 minutes. A red color indicates a positive reaction (NO_2). A negative reaction (yellow) may be due to the reduction to nitrogen. Add 2 to 3 mg of Zn dust to the GLU tube. After 5 minutes, if the tube remains yellow this indicates a positive reaction (N_2). If the test turns orange-red, this is a negative reaction.

8.7.5.2. Identify the microorganism by entering the 9-digit numerical profile manually using the APIweb software.

9. History:

Name	Date	Amendment
Melanie Lenahan	29MAY07	Initial Release
Sheila Byrne	22MAY08	Revision 1
Sheila Byrne	19JUL10	Revision 2
Jason McMillan	18MAR14	College name change, added Reading Table

SOP: API 20E Microbial Identification

10. Attachments:

Figure 1: Reading Table

TESTS	ACTIVE INGREDIENTS	QTY (mg/cup.)	REACTIONS/ENZYMES	RESULTS	
				NEGATIVE	POSITIVE
ONPG	2-nitrophenyl-βD-galactopyranoside	0.223	B-galactosidase (Ortho NitroPhenyl-βD-Galactopyranosidase	colorless	yellow (1)
ADH	L-arginine	1.9	Arginine DiHydrolase	yellow	red/orange (2)
LDC	L-lysine	1.9	Lysine DeCarboxylase	yellow	red/orange (2)
ODC	L-ornithine	1.9	Ornitithine DeCarboxylase	yellow	red/orange (2)
CIT	Trisodium citrate	0.756	CITrate utilization	pale green/yellow	blue-green/blue (3)
H_2S	sodium thiosulfate	0.075	H_2S production	colorless/greyish	black deposit/thin line
URE	urea	0.76	UREase	yellow	red/orange (2)
TDA	L-tryptophane	0.38	Tryptophane DeAnimase	TDA/immediate	
				yellow	reddish brown
IND	L-tryptophane	0.19	INDole Production	James/immediate	
				colorless pale green/yellow	pink
VP	sodium pyruvate	1.9	acetoin production (Vogues Proskauer)	VP1 + VP2/10 min	
				colorless	pink/red (5)
GEL	Gelatin (bovine origin)	0.6	GELatinase	no diffusion	diffusion of black pigment
GLU	D-glucose	1.9	fermentation/oxidation (GLUcose) (4)	blue/blue-green	yellow/greyish yellow
MAN	D-mannitol	1.9	fermentation/oxidation (MANnitol) (4)	blue/blue-green	yellow
INO	onositol	1.9	fermentation/oxidation (INOsitol) (4)	blue/blue-green	yellow
SOR	D-sorbitol	1.9	fermentation/oxidation (SORbitol) (4)	blue/blue-green	yellow
RHA	L-rhamnose	1.9	fermentation/oxidation (RHAmnose) (4)	blue/blue-green	yellow
SAC	D-sucrose	1.9	fermentation/oxidation (SACcharose) (4)	blue/blue-green	yellow
MEL	D-melibiose	1.9	fermentation/oxidation (MELibiose) (4)	blue/blue-green	yellow
AMY	amygdalin	0.57	fermentation/oxidation (AMYgdalin) (4)	blue/blue-green	yellow
ARA	L-arabinose	1.9	fermentation/oxidation (ARAbinose) (4)	blue/blue-green	yellow
OX	(see oxidase test package insert)		cytochrome-Oxidase	(see oxidase test package insert)	

(1) A very pale yellow should also be considered positive.
(2) An orange color after 36-48 hours incubation must be considered negative.
(3) Reading made in the cupule (aerobic).

SOP: API 20E Microbial Identification

(4) Fermentation begins in the lower portion of the tubes, oxidation begins in the cupule.
(5) A slightly pink color after 10 minutes should be considered negative.
* The quantities indicated may be adjusted depending on the titer of the raw materials used.
* Certain cupules contain products of animal origin, notably peptones.

SOP: Mycoplasma Testing

Approvals:

Preparer: Dr. Maggie Bryans — Date: 17FEB14
Reviewer: Jason McMillan — Date: 19FEB14

1. **Purpose:**
   1.1. Testing of samples for presence of mycoplasma.
2. **Scope:**
   2.1. Applies to testing of solutions including media and cultures for presence of mycoplasma.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Takara mycoplasm testing protocol
   4.2. Amersham Bioscience puReTaq Ready to go PCR beads protocol
   4.3. thermocycler SOP
   4.4. gel documentation System SOP
5. **Definitions:** N/A
6. **Precautions:**
   6.1. Ethidium Bromide is a mutagen. Use care and wear double gloves.
   6.2. UV light can damage eyes, wear UV shields when using the UV light box
7. **Materials:**
   7.1. Takara Mycoplasma Detection PCR Kit (Catalog number: 6601)
      7.1.1. Takara forward primer diluted 1:5 with PCR grade water
      7.1.2. Takara reverse primer diluted 1:5 with PCR grade water
      7.1.3. Takara control template diluted 1:5 with PCR grade water
   7.2. Amersham Bioscience pure Taq Ready to Go PCR beads (Catalog number: 27-9557-01)
   7.3. PCR grade water
   7.4. mineral oil
   7.5. agarose
   7.6. 10x TBE buffer
   7.7. 1% ethidium bromide solution
   7.8. DNA sample buffer
   7.9. 1kb DNA ladder (New England Biolabs, Catalog number: 3232L)
   7.10. sterile filter pipet tips (0-30µL and up to 200µL)
   7.11. thermal cycler
   7.12. horizontal electrophoresis box
   7.13. power Supply
   7.14. gel documentation system with UV light
8. **Procedure:**
   8.1. **PCR amplification**
      8.1.1. **Preparation of samples**

SOP: Mycoplasma Testing

8.1.1.1. Verify that a bead is visible at the bottom of each tube. If necessary tap the tube against a hard surface to force the bead to the bottom of the tube.

8.1.1.2. Label tubes as: sample, +C1, +C2, -C
Write the date and your initials on all tubes.

8.1.1.3. Add the following to each tube containing a PCR bead:

	Sample	+ Control **1**	+ Control **2**	- Control
Sterile distilled water	18µL	22µL	17µL	23µL
Forward primer	1µL	1µL	1µL	1µL
Reverse primer	1µL	1µL	1µL	1µL
Control DNA	n/a	1µL	1µL	n/a
Sample DNA	5µL	n/a	5µL	n/a

8.1.1.4. Mix the contents of the tubes by gently pipetting up and down.

8.1.1.5. Overlay each reaction mix with 50µL mineral oil.

8.1.2. **Amplification of samples**

8.1.2.1. Turn on instrument.

8.1.2.2. Verify wells in the thermocycler have mineral oil and add mineral oil if needed.

8.1.2.3. Verify that PCR program number 47 has not been changed.
Program should be:

94C	5min	1 cycle
94C	1min	\
55C	2min	35 cycles
72C	1min	/
4C	10min	

If necessary, edit the program.

8.1.2.4. Place all tubes in the thermal cycler.

8.1.2.5. Start program number 47.

8.1.2.6. When program is complete, shut off instrument and remove tubes.

8.1.2.7. Store tubes at -20°C.

8.2. **Analysis by Electrophoresis**

8.2.1. **Preparation of 1x TBE Running Buffer**

8.2.1.1. Dilute the 10x TBE to 1x TBE by combining 100mL 10x TBE with 900mL DI water. Mix well.

8.2.2. **Preparation of 2% Agarose Gel**

8.2.2.1. Assemble gel box WITHOUT the comb.

8.2.2.2. Weigh out 2 ±0.1 grams of agarose and place into a 250mL Erlenmeyer flask.

8.2.2.3. Add 100mL 1xTBE buffer to the agarose.

8.2.2.4. Microwave the agarose mixture until it begins to boil (~3 minutes).

8.2.2.5. Carefully remove from microwave and swirl to mix.

8.2.2.6. Pour agarose mix into the gel box tray until the height of the agarose mix is approximately 0.5cm.

8.2.2.7. Add 10µL ethidium bromide and mix with the pipet tip.

SOP: Mycoplasma Testing

Reminder: Ethidium bromide is a mutagen, use care when handling and wear double gloves.

8.2.2.8. Place the comb into the tray.
8.2.2.9. Allow the gel to set (approximately 30 minutes).
8.2.2.10. When the gel is set, remove the comb, reverse the tray and add enough 1X TBE buffer to just cover the gel.

8.2.3. **Running the Gel**

8.2.3.1. Remove PCR tubes from the -20°C freezer and IMMEDIATELY remove as much mineral oil from the top as soon as possible before the samples thaw.
8.2.3.2. Add 10µL sample buffer to each tube and mix well.
8.2.3.3. Load 15µL of each sample into individual wells. Record position of samples.
8.2.3.4. Load 10µL of DNA ladder into an adjacent well. Record position of ladder.
8.2.3.5. Connect the lid to the electrophoresis apparatus.
8.2.3.6. Connect the leads to the power supply making sure to use the color codes.
8.2.3.7. Turn on the power supply and run at 100Vfor approximately 1-2 hours.
8.2.3.8. Turn off the power supply.

8.2.4. **Analysis**

8.2.4.1. Wearing double gloves, carefully remove gel and tray from the apparatus and place into a storage container designated for ethidium bromide.
8.2.4.2. Observe under the UV light box.

Reminder: Use the UV shield.

8.2.4.3. Take a photograph of the gel.
8.2.4.4. Molecular weight of the + Control PCR band should be 810bp.

9. Attachments:

9.1. Data Table
9.2. Picture of 1kb DNA Ladder

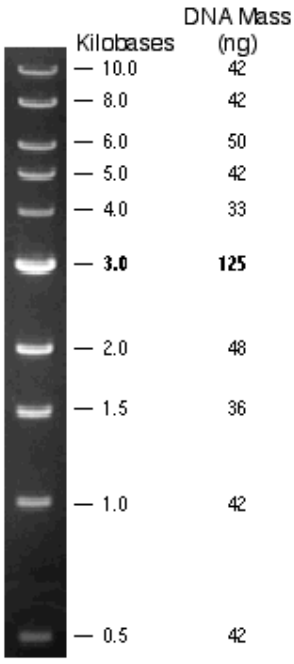
10. History:

Name	Date	Amendment
Deb Audino	02Feb05	Initial Release
Deb Audino	10Oct05	Added catalog number for the Takara kit. Added concentration of ethidium bromide stock solution. Reduced volume of ethidium bromide solution for gel. Added attachments.
Deb Audino	04Apr08	College name change
Jason McMillan	19Feb14	College name change

Lane	ID of PCR	Presence (+) or

SOP: Mycoplasma Testing

Position	reaction loaded	Absence (-) of 810bp band
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		



1kb DNA Ladder (New England Biolabs)

UPSTREAM PROCESSING: CHO CELL TPA

SOP: Labconco Purifier Class 2 Biological Safety Cabinet (BSC) Operation

Approvals:
Preparer: Jason McMillan Date: 17JAN14
Reviewer: Dr. Margaret Bryans Date: 18JAN14

1. **Purpose:**
   1.1. Operation of the Biological Safety Cabinet (BSC).
2. **Scope:**
   2.1. Applies to the use of the BSC for maintaining a sterile environment for media preparation, culture inoculation and culture sampling.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Labconco Purifier Class 2 Biological Safety Cabinet Operator's Manual
5. **Definitions:** N/A
6. **Precautions:**
   6.1. UV Light is damaging to eyes and skin. Avoid exposure.
7. **Materials:**
   7.1. 70% Isopropanol (IPA) in spray bottle
   7.2. lab towels
   7.3. lab coat
   7.4. gloves
8. **Procedure:**
   8.1. UV Decontamination – Performed for initial use of the day
   8.1.1. Put on gloves and lab coat.
   8.1.2. Open sash **slightly** and **immediately** turn on blower.
   8.1.3. Spray and wipe down the stainless steel work surfaces of the BSC with 70% IPA.
   8.1.4. With gloved hands, spray all necessary materials not affected by UV with 70% IPA and place in BSC.
   8.1.5. Close the sash, turn off the blower, and turn on the UV light. This switch is located on the control panel. Refer to Figure 1.
   8.1.6. Leave the UV light on for at least 15 minutes.
   8.1.7. Place a biohazard waste receptacle adjacent to the cabinet.
   8.1.8. Once the appropriate time has elapsed, turn off the UV light.
   8.1.9. Turn on the visible light. This is located on the control panel.
   8.1.10. Turn on the receptacle power.
   8.1.11. Open the sash **slightly,** and **immediately** turn on the blower.
   8.1.12. After blower is on, raise the sash to the safe operating level indicated by a red dot on the left side of the cabinet.

SOP: Labconco Purifier Class 2 Biological Safety Cabinet (BSC) Operation

Note: The alarm will sound if the sash is raised above this level.

8.2. Operation

8.2.1. Replace gloves with new ones.

8.2.2. Spray down hands with 70% IPA prior to entering the BSC.
Note: Allow 30 seconds for the 70% IPA to dry. This ensures disinfection.

8.2.3. Spray all necessary equipment that needs to go into the BSC with 70% IPA, and allow to dry for 30 seconds.

8.2.4. Place all necessary equipment inside of the BSC.

8.2.5. Perform protocol while working in center of the work surface.
Note: **Do not block the intake grills**. This ensures proper airflow. Refer to Figure 3.

8.2.6. Once the protocol is completed, remove all equipment from the BSC.

8.2.7. Place any disposable materials that have contacted any cellular organism into the biohazard waste receptacle.

8.2.8. Spray down the stainless steel work surfaces with 70% IPA. Allow 30 seconds for the IPA to dry, then wipe down stainless steel work surfaces with a lab towel. Spray down the work surfaces once more, but allow the IPA to dry without wiping.

8.2.9. Dispose lab towels used to clean BSC into the biohazard waste receptacle.

8.2.10. Turn off the BSC.

9. Attachments:

SOP: Labconco Purifier Class 2 Biological Safety Cabinet (BSC) Operation

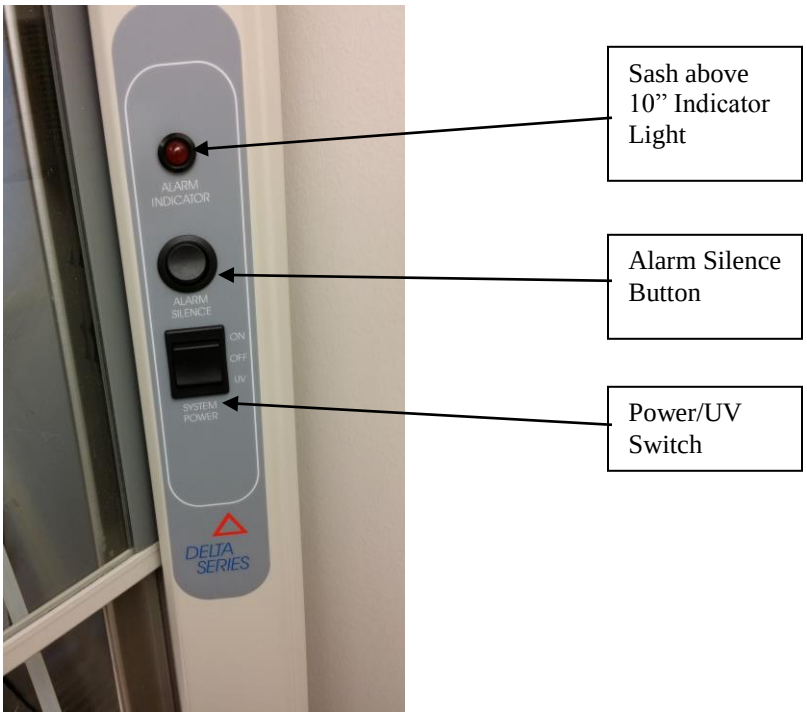
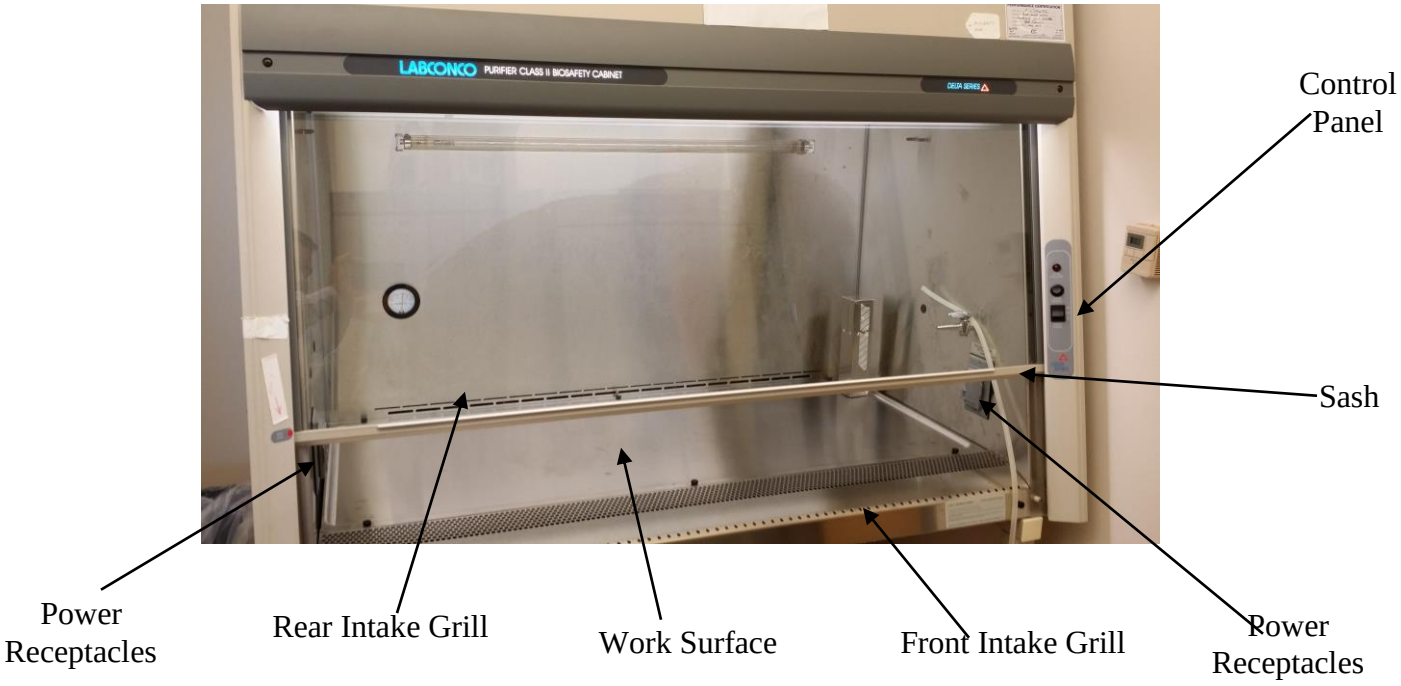


Figure 1: Biological Safety Cabinet Control Panel



SOP: Labconco Purifier Class 2 Biological Safety Cabinet (BSC) Operation

Figure 2: Biological Safety Cabinet Components

Figure 3: Air Flow Diagram
http://www.labconco.com/product/purifier-logic-class-ii-type-a2-biosafety-cabinets-2/4261

10. History:

Revision Number	*Effective Date*	*Preparer*	*Description of Change*
0	17JAN14	Jason McMillan	Initial release

Montgomery County Community College
340 DeKalb Pike
Blue Bell, PA 19422

SOP: Bellco Spinner Flask (100mL) Cleaning and Autoclaving

Approvals:

Preparer: Dr. Maggie Bryans Date: 03APR09
Reviewer: Jason McMillan Date: 03APR09

1. **Purpose:**
   1.1. Cleaning and assembling of the Bellco Spinner Flask (100mL).
2. **Scope:**
   2.1. Applies to the Bellco Spinner Flask (100mL) for maintaining suspension of cultures.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that the SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1.1. Bellco Adjustable Hanging Bar Spinner Flask (100mL) manufacturer insert
   4.1.2. Autoclave SOP
   4.1.3. Bellco Micro-Carrier Spinner Flasks (500mL) SOP
   4.1.4. http://www.bellcoglass.com/
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. magnetic stirrer plate
   7.2. glassware detergent
   7.3. bottle brush
   7.4. autoclave
8. **Procedure:**
   8.1. **Preparation**
      8.1.1. Gather all parts: glass spinner flask body, sidearm caps, top cap, compression fitting body, compression fitting retaining nut, cap liner, shaft lock (cap nut, washer and o-ring), glass impeller shaft, magnet and magnet holder.
      8.1.2. Wash all parts with a bottle brush, warm water and glassware detergent. Rinse several times with tap water, and then several times with deionized water.
   8.2. **Assembly**
      8.2.1. Insert the glass impeller shaft into the hole at the bottom of the magnet holder. The knob at the bottom of the shaft will catch and the magnet holder should spin freely.
      8.2.2. Insert magnet into the remaining holes on the magnet holder so that it is centered across the bottom.
      8.2.3. Insert cap liner into the top cap. Push the compression fitting body up through the hole in the bottom of the top cap and lock in place using the retainer nut on the topside of the top cap.
      8.2.4. Gently slide the glass impeller shaft through the cap from the bottom. Slide the o-ring onto the shaft so that it is flush with the cap liner.

SOP: Bellco Spinner Flask (100mL) Cleaning and Autoclaving

8.2.5. Insert the impeller assembly into the glass spinner flask body and screw on the top cap.
8.2.6. Adjust the height of the impeller assembly by sliding the glass shaft up or down so that the magnet hangs just above the floor of the glass spinner flask body.
8.2.7. Slide the cap nut onto the glass impeller shaft on top of the washer and tighten.
8.2.8. Test the behavior of the impeller assembly by placing the assembled flask on a magnetic stir plate, and adjusting the height of the glass impeller shaft until the impeller spins properly.
8.2.9. Screw on the side arm caps.
8.3.1. Autoclave per autoclave SOP before and after use. (Remember to loosen side arm caps prior to autoclaving).

9. Attachments:

9.1. Figure 1: Spinner Flask Cap Assembly (http://www.bellcoglass.com/)
9.2. Figure 2: Spinner Flask Impeller Assembly (http://www.bellcoglass.com/)

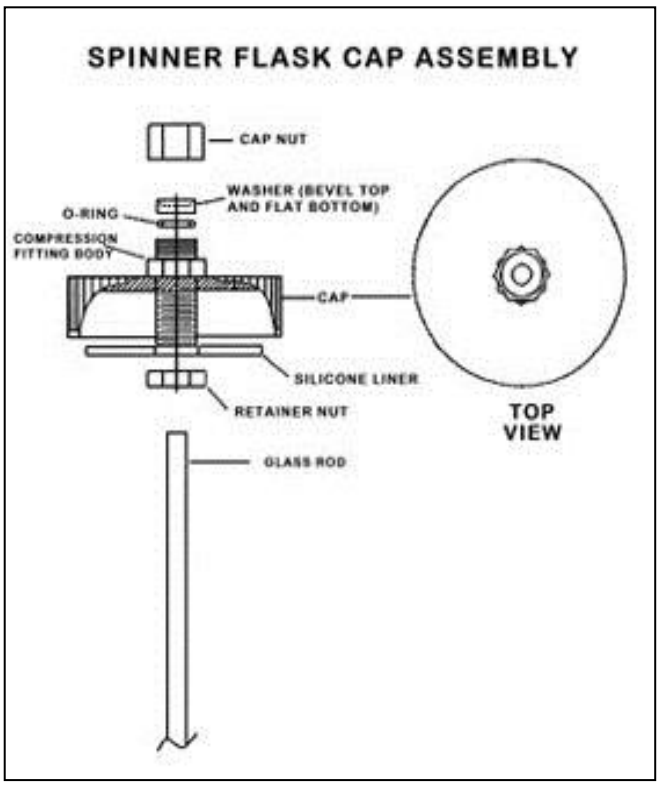


Figure 1: Spinner Flask Cap Assembly
(http://www.bellcoglass.com/)

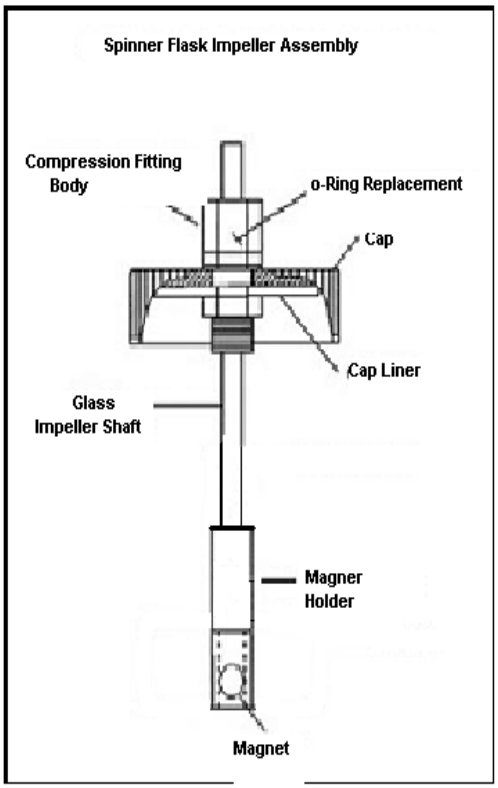


Figure 2: Spinner Flask Impeller Assembly
(http://www.bellcoglass.com/)

SOP: Bellco Spinner Flask (100mL) Cleaning and Autoclaving

10. History:

Name	Date	Amendment
Kari Britt Katrice Jalbert	03FEB06	Initial release
Deb Audino	04APR08	College name change
Kari Britt	03APR09	Formatting and clarification to directions
Jason McMillan	18MAR14	College name change

SOP: Batch Culture of Recombinant tPA Secreting CHO Cells

Approvals:

Preparer: Jason McMillan — Date 19MAR14
Reviewer: Dr. Margaret Byans — Date 20MAR14

1. **Purpose:**
   1.1. To produce a batch culture of mammalian cells.
2. **Scope:**
   2.1. Applies to the production of human tissue plasminogen activator (tPA) protein from recombinant Chinese Hamster Ovary (CHO) cells.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. ATCC CRL9606 growth guidelines
   4.2. Bellco Spinner Flask (100mL) Cleaning and Autoclaving SOP
   4.3. Labconco Purifier Class 2 Biological Safety Cabinet (BSC) Operation SOP
   4.4. CO_2 Incubator SOP
   4.5. Oakton PC 700 Bench Series pH/ Conductivity/°C/°F Meter SOP
   4.6. spectrophotometer SOP
   4.7. Glucose Determination Assay SOP
   4.8. Lactate Determination Assay SOP
   4.9. Trypan Blue Assay SOP
   4.10. Human tPA Total Antigen ELISA SOP
   4.11. Human tPA Activity ELISA SOP
   4.12. Applikon ez-Control Bioreactor Controller Operation SOP
5. **Definitions:** N/A
6. **Precautions:**
   6.1. Use BL2 safety measures and discard waste in biohazard containers.
7. **Materials:**
   7.1. biological safety cabinet
   7.2. vial of CHO cells (ATCC 9606-CRL) recombinant for human tissue plasminogen activator (tPA)
   7.3. Ham's F12 Medium
   7.4. fetal bovine serum (FBS)
   7.5. 10X PBS
   7.6. 100mL vessel
   7.7. 1M $NaHCO_3$ (sodium bicarbonate)
   7.8. 200mM glutamine
   7.9. 10mg/mL gentamycin
   7.10. sterile 100mL Bellco spinner flasks
   7.11. sterile transfer pipets (2mL, 5mL, 25 ml, and 50mL) and pipette aid

SOP: Batch Culture of Recombinant tPA Secreting CHO Cells

7.12. CO_2 incubator containing magnetic stir plate
7.13. UV-visible recording spectrophotometer
7.14. cuvettes for spectrophotometer
7.15. cuvette rack
7.16. 15ml conical tubes
7.17. Test tube rack
7.18. 1.5mL microfuge tubes
7.19. Microfuge tube holder
7.20. P20 and P1000 micropipette
7.21. microscope with 1000x magnification
7.22. cryogenic vials (1mL capacity) for storage of CHO cell master/working cell bank
7.23. sterile 250mL glass bottles for storage of CHO cell media
7.24. 100 mL glass bottle
7.25. 1L addition bottle
7.26. Male and female autoclavable connectors

8. Procedure:

8.1. **Initial Media Preparation:** Ham's F12 Medium, 90%; Fetal Bovine Serum, 10%:

8.1.1. Clean, assemble, and autoclave 100mL Bellco spinner flasks per SOP.

8.1.2. Gather the following items, spray with 70% isopropanol, and place in the biological safety cabinet:
(1) Pipette aid
(2) 5mL sterile pipettes
(2) 25mL sterile pipettes
(2) 100mL sterile Bellco spinner flasks
(1) 500mL bottle of pre-sterilized Ham's F12 Medium
(1) 50mL tube of pre-sterilized, heat inactivated fetal bovine serum (FBS)

8.1.3. Prepare biological safety cabinet (BSC) per Labconco Purifier Class 2 Biological Safety Cabinet (BSC) Operation SOP.

8.1.4. Sterilely remove 21.6mL of Ham's F12 Medium from a 500mL bottle of Ham's F12 and add to a sterile 100mL spinner flask.
8.1.4.1. Repeat with a second 100mL spinner flask.

8.1.5. Sterilely add 2.4mL of FBS to the sterile 100mL spinner flask.
8.1.5.1. Repeat with the second 100mL spinner flask.

8.1.6. Label one spinner flask as 90% Ham’s F12, 10% FBS, [date], [group#], [operator initials]. Label the second spinner flask as **BLANK**, [date], [group#], [operator initials]

8.1.7. Place all spinner flasks containing CHO cell media in the CO_2 incubator. Set the speed of the magnetic stirrer to 60 rpm to ensure an even mixing of the culture without foaming.
8.1.7.1. Verify that the temperature is 37 ± 0.5°C and percentage of CO_2 is 5 ± 0.5%.

8.1.8. Check media for contamination after a minimum of 24 hours.

8.2. **Inoculation**

SOP: Batch Culture of Recombinant tPA Secreting CHO Cells

8.2.1. Spray two 2mL sterile pipettes and a pipette aid with 70% isopropanol and place in the BSC.
8.2.2. Prepare Biological Safety Cabinet per BSC SOP.
8.2.3. Remove two vials of CHO cells from storage in the -80°C freezer and note in the ScienTemp -80°C Freezer Log.
8.2.4. Thaw contents rapidly by agitation in a 37°C ± 0.5°C water bath.
8.2.5. Spray vials with 70% isopropanol, and place in the biological safety cabinet.
8.2.6. Sterilely transfer the entire contents of both 1mL vials of thawed CHO Cells into the Bellco Spinner Flask labeled 90% Ham's F12, 10% FBS, [date], [group#], [operator initials] using a 2mL sterile pipet. **Do not add anything to the Bellco Spinner Flask labeled BLANK, [date], [group#], [operator initials].**
8.2.7. Swirl to mix.
8.2.8. Immediately after adding CHO Cells to Bellco Spinner Flask labeled 90% Ham's F12, 10% FBS, [date], [group#], [operator initials] using a 2mL sterile pipet (day 0) and at 1-day intervals the culture will be sampled to determine the OD, pH, viable cell count, analyte levels and tPA concentration. The culture will be scaled up just before the exponential phase of the growth curve begins to slow down, indicating the cell culture is moving into the stationary phase of the growth curve. The live cell concentration should be approaching 1 million cells/ml.

8.3. **Sampling the Culture**

8.3.1. Turn on the spectrophotometer to allow the lamp to warm up for use.
8.3.2. Collect the following items:
(4) microfuge tubes labeled "tPA" and "cells" and "trypan" and "microcentrifuge counterbalance"
(1) microfuge tube holder
(2) spectrophotometers cuvettes (1 labeled "Sample" and 1 labeled "Blank")
(1) cuvette holder
(1) P1000 pipette
(1) P20 pipette
(1) pipette aid
8.3.3. Prepare biological safety cabinet per Labconco Purifier Class 2 Biological Safety Cabinet (BSC) Operation SOP.
8.3.4. Collect the following items, spray with 70% IPA and place in Biological Safety Cabinet:
(1) 15ml conical tube
(1) test tube rack
(1) cuvette
(1) cuvette rack
(1) pipette aid
(1) 5mL pipette
(1) 2mL pipettes
8.3.5. Prepare pH Meter per Oakton PC 700 Bench Series pH/ Conductivity/°C/°F Meter SOP.
8.3.6. Prepare spectrophotometer per spectrophotometer SOP using media from the BLANK, [date], [group#], [operator initials] Bellco Spinner Flask to zero the machine.

SOP: Batch Culture of Recombinant tPA Secreting CHO Cells

8.3.7. Spray BLANK, [date], [group#], [operator initials] Bellco Spinner Flask and 90% Ham's F12, 10% FBS, [date], [group#], [operator initials] Bellco Spinner Flask with 70% IPA and place in biological safety cabinet.

8.3.8. Using aseptic technique, remove 3 mL from 90% Ham's F12, 10% FBS, [date], [group#], [operator initials] Bellco Spinner Flask and place into a 15ml conical tube. Using aseptic technique, remove 1 mL from BLANK, [date], [group#], [operator initials] Bellco Spinner Flask and place into a cuvette.

8.3.9. Remove all items from the biological safety cabinet.

8.3.10. Return 90% Ham's F12, 10% FBS, [date], [group#], [operator initials] Bellco Spinner Flask and BLANK, [date], [group#], [operator initials] Bellco Spinner Flask to the CO2 incubator, making sure to loosen side arm caps once in incubator.

8.3.11. Cover the blank cuvette with Parafilm.

8.3.12. Remove 1ml of sample from 15ml conical tube and place in cuvette labeled "Sample."

8.3.13. Take OD Reading at 650nm per spectrophotometer SOP.

8.3.13.1. Mix CHO sample by inverting the cuvette several times before taking reading.

8.3.13.2. After reading return to 15ml conical tube for pH measurement.

8.3.14. Remove 100µl from 15ml conical tube and place in microfuge tube labeled "cells."

8.3.15. Determine cell count using the Trypan Blue SOP.

8.3.16. Using the 2.9ml in the conical tube take the pH reading per Oakton PC 700 Bench Series pH/ Conductivity/°C/°F Meter SOP.

8.3.17. Remove 1ml of sample and place in a 1.5mL tube and centrifuge in the benchtop mini centrifuge for 5minutes being sure to counterbalance the sample microfuge tube with the "microcentrifuge counter balance" microfuge tube containing 1ml of water. Remove the supernatant and place in the microfuge tube labeled "tPA," and label with Date, Group Name, and Vessel Name. Store at 2-8°C in a microfuge tube storage box labeled with Date, Group Name, Vessel Name until needed.

8.3.18. Record all sample data in batch record.

8.4. **Scale up to 100ml of media in 90% Ham's F12, 10% FBS, [date], [group#], [operator initials] Bellco Spinner**

8.4.1. Gather the following items, spray with 70% isopropanol, and place in the biological safety cabinet:
(1) Pipette aid
(2) 10mL sterile pipettes
(4) 50mL sterile pipettes
90% Ham's F12, 10% FBS, [date], [group#], [operator initials] Bellco Spinner Flask
(1) 500mL bottle of pre-sterilized Ham's F12 Medium
(1) 50mL tube of pre-sterilized, heat inactivated fetal bovine serum (FBS)

8.4.2. Sterilely remove 74.7mL of Ham's F12 Medium from a 500mL bottle of Ham's F12 and add to 90% Ham's F12, 10% FBS, [date], [group#], [operator initials] Bellco Spinner Flask.

8.4.3. Sterilely remove and add 8.3mL of FBS to 90% Ham's F12, 10% FBS, [date], [group#], [operator initials] Bellco Spinner Flask.

8.4.4. Return 90% Ham's F12, 10% FBS, [date], [group#], [operator initials] Bellco Spinner

SOP: Batch Culture of Recombinant tPA Secreting CHO Cells

Flask to the CO2 incubator, making sure to loosen side arm caps once in incubator.

8.5. **Scale up to 1L bioreactor**

Note: When the 100mL suspension culture of CHO cells reaches a concentration of approximately 1,000,000 cells/mL, the entire contents of the 100mL spinner flask will be added to the bioreactor containing 1L of CHO cell growth media.

8.5.1. Prepare 1M $NaHCO_3$ (sodium bicarbonate)

8.5.1.1. Weigh out 21 ± 1 grams of $NaHCO_3$ and transfer to an Applikon bioreactor feed bottle.

8.5.1.2. Label the bottle as 1M $NaHCO_3$, [date], [initials], [group number], storage: room temp, disposal: drain.

8.5.1.3. Using a 250mL graduated cylinder, measure 250 ± 5mL deionized water and transfer into the feed bottle.

8.5.1.4. Add a magnetic stir bar and stir on a magnetic stirrer to dissolve.

8.5.1.5. Remove the stir bar and add lid and tubing per Applikon ez-Control Bioreactor Controller Operation SOP.

8.5.2. Prepare the Applikon bioreactor and 1L addition bottle with tubing and autoclavable male connector attached for autoclaving per the Applikon ez-Control Bioreactor Controller Operation SOP including calibrating the pH probe.

8.5.3. Autoclave the Applikon bioreactor with 100ml of 1X PBS and 1L addition bottle with tubing and autoclavable male connector attached per the Applikon ez-Control Bioreactor Controller Operation SOP.

8.5.4. Remove the Applikon bioreactor vessel from the autoclave and connect the DO probe to the controller.

8.5.5. Gather the following items, spray with 70% isopropanol, and place in the biological safety cabinet:
(1) Pipette aid
(1) 10mL sterile pipette
(2) 50mL sterile pipettes
(2) 500mL bottle of pre-sterilized Ham's F12 Medium
(1) 50mL tube of pre-sterilized, heat inactivated fetal bovine serum (FBS)
(1) 10mL bottle of 10mg/mL gentamycin
(1) empty 50ml tube
1L addition bottle with tubing and autoclavable male connector attached

8.5.6. Aseptically remove 50ml from one 500mL bottle of pre-sterilized Ham's F12 Medium and place in the empty 50ml tube.

8.5.7. Aseptically add the remaining 450ml and an additional 500mL bottle of pre-sterilized Ham's F12 Medium to the 1L addition bottle with tubing and autoclavable male connector attached.

8.5.8. Aseptically add 50ml of FBS to the 1L addition bottle with tubing and autoclavable male connector attached.

8.5.9. Aseptically add the 10mL bottle of 10mg/mL gentamycin.

8.5.10. Be sure the cap is on tightly and remove the 1L addition bottle with tubing and autoclavable male connector attached and bring it over to the Applikon bioreactor.

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8.5.11. Carefully remove the foil from the female connector on the addition port of the Applikon bioreactor.
8.5.12. Carefully remove the aluminum foil from the male connector on the 1L addition bottle and connect the male connector to the female connector on the addition port of the bioreactor.
8.5.13. Remove the clamp on the female connector on the addition port of the Applikon bioreactor.
8.5.14. On the Applikon touch screen select Menu > Manual Control > Acid Pump On
8.5.15. As the pump turns feed the tubing around it. Use care to avoid pinching fingers.
8.5.16. Once all of the media has transferred into the vessel turn off the acid pump. On the Applikon touch screen select Menu > Manual Control > Acid Pump Off
8.5.17. Disconnect the male connector of the addition bottle from the female connector on the addition port of the bioreactor. Bend the tubing of the addition port and reattach the clamp.
8.5.18. Connect the remaining parts of the bioreactor to the controller.
8.5.19. Input the setpoints and limits listed in the table below per the bioreactor SOP.

Parameter	pH	Temp (^{o}C)	%DO	Stirrer (rpm)
Set point	7.2	37	50	75
Upper Limit	7.3	38	52	76
Lower Limit	7.1	36	48	74

8.5.20. Allow the DO probe to polarize for a minimum of 6 hours.
8.5.21. Calibrate the DO probe per the Applikon ez-Control Bioreactor Controller Operation SOP.
8.5.22. Immediately (Day 0) and at 1-day intervals, sample the culture to determine OD, pH, viable cell count, analytes and tPA over time (see step 8.7).

8.6. When the cell count reaches approximately 1,000,000 cells/mL shut down the bioreactor per the Applikon ez-Control Bioreactor Controller Operation and harvest cells as described below.

8.7. **Bioreactor Sampling Instructions Days 0-2**

8.7.1. Log in as operator if not already done.
8.7.2. Raise the stirrer upper limit to 150 rpm.
8.7.3. Change the stirrer setting to 125 rpm.
8.7.4. Spray the headplate near the sampling tube with 70% IPA.
8.7.5. Remove the black clamp and set on the head plate.
8.7.6. Pull out the autoclavable female connector and set it next to the black clamp.
8.7.7. Place a 50ml pipette into the sampling tube and remove 50ml of sample and place in a 50ml tube.
8.7.8. Put the female autoclavable connector back into the sampling tube.
8.7.9. Bend the sampling tubing and place the black clamp back on the tubing.
8.7.10. Change the stirrer setting to 75 rpm.
8.7.11. Change the stirrer upper limit back to 76 rpm.
8.7.12. Testing preparation
8.7.12.1. Remove 1ml for OD and return back to the 50ml tube.

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8.7.12.2. Spin the 50ml tube at 900 rpm for 5 minutes.
8.7.12.3. Remove 1 ml of supernatant and store with previous tPA samples.
8.7.12.4. Remove all of the supernatant being sure not to disturb the pellet.
8.7.12.5. Re-suspend the pellet in 1ml of excess supernatant using a 5ml pipette. Discard the additional supernatant.
8.7.12.6. Perform the trypan blue assay per SOP.

8.8. **Bioreactor Sampling Instructions Days 3-EOR**
8.8.1. Log in as operator if not already done.
8.8.2. Raise the stirrer upper limit to 150 rpm.
8.8.3. Change the stirrer setting to 125 rpm.
8.8.4. Spray the headplate near the sampling tube with 70% IPA.
8.8.5. Remove the black clamp and set on the head plate.
8.8.6. Pull out the autoclavable female connector and set it next to the black clamp.
8.8.7. Place a 5ml pipette into the sampling tube and remove 5ml of sample and place in a 15ml tube.
8.8.8. Put the female autoclavable connector back into the sampling tube.
8.8.9. Bend the sampling tubing and place the black clamp back on the tubing.
8.8.10. Change the stirrer setting to 75 rpm.
8.8.11. Change the stirrer upper limit back to 76 rpm.
8.8.12. Perform testing per SOP's.

8.9. **Determine tPA Concentration**
8.9.1. Determine the tPA concentration at each time point per Human tPA Total Antigen ELISA SOP.
8.9.2. Determine the activity of the tPA at each time point per Human tPA Activity ELISA SOP.

8.10. Determine Lactate Concentration
8.10.1. Determine the lactate concentration at each time point per the Lactate Determination Assay SOP.

8.11. Determine Glucose Concentration
8.11.1. Determine the glucose concentration at each time point per the Glucose Determination Assay SOP.

8.12. **Prepare Growth Curves**
8.12.1. Plot OD, pH, viable cells, glucose, lactate, and tPA vs. time (use 2 y-axes).
8.12.2. Attach growth curve to Batch Record.
8.12.3. Determine growth rate and doubling time of the 100mL spinner flask and 1L bioreactor cultures.
8.12.4. Attach calculations to Batch Record.

9. Attachments:
9.1. Data table

10. History:

Name	Date	Amendment
Jason McMillan	18Mar14	Initial Release
Jason McMillan	26JUN15	Extensive modification for optimization

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The following parameters are recorded in the batch record doc #....

TIME (hours)	OD 650nm	pH	LIVE Cell Count	DEAD Cell Count	Viable Cells/mL	Percent Viability	GLU mg/dL	LAC mmol/L

SOP: Applikon ez-Control Bioreactor Controller Operation

Approvals

Preparer: John Buford — Date: 19DEC13
Reviewer: Jason McMillan — Date: 20DEC13
Reviewer: Dr. Margaret Bryans — Date: 21DEC13

1. Purpose

1.1. Cultivate a cell culture using an Applikon ez-Control bioreactor controller and a 3-liter glass autoclavable bioreactor.

2. Scope and Applicability

2.1. A bioreactor controller is used to measure and control process variables (temperature, pH, dissolved oxygen, stirrer speed, and so on) within a bioreactor vessel such as a glass autoclavable bioreactor, a single use bioreactor, or a single use wave bag. This SOP provides the basic steps required to cultivate a cell culture using the Applikon ez-Control and a 3-liter glass autoclavable bioreactor. Other process SOPs are intended to provide additional details such as culture medium composition and volume, control process settings, and run time.

3. Summary of Method

3.1. Preparing the controller
- 3.1.1. Power up the controller and login
- 3.1.2. Enter the project name (optional)
- 3.1.3. Emergency stop and resume (emergency only)

3.2. Preparing the bioreactor
- 3.2.1. Fill the bioreactor with culture medium
- 3.2.2. Calibrate the pH sensor
- 3.2.3. Mount sensors to the bioreactor
- 3.2.4. Prepare the liquid addition bottles
- 3.2.5. Mount connections to the bioreactor
- 3.2.6. Autoclave the bioreactor and addition bottles

3.3. Connecting the bioreactor to the controller
- 3.3.1. Verify that all control loops are switched off
- 3.3.2. Connect the sensors
- 3.3.3. Connect the heating blanket
- 3.3.4. Connect the aeration
- 3.3.5. Connect the stirrer motor
- 3.3.6. Connect the alkaline bottle
- 3.3.7. Enter process parameter settings
- 3.3.8. Start all control loops
- 3.3.9. Calibrate the DO sensor
- 3.3.10. Reset dose monitor values

3.4. Starting the cultivation
- 3.4.1. Start process data acquisition
- 3.4.2. Inoculate the bioreactor

3.5. Monitoring the cell culture

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3.5.1. View process data
3.5.2. Sample the cell culture
3.6. Harvesting the cell culture
3.6.1. Stop the controllers
3.6.2. Save process data
3.6.3. Disconnect the bioreactor
3.6.4. Decant the cell culture
3.7. Cleaning the bioreactor
3.7.1. Clean the sensors
3.7.2. Pre-clean the bioreactor in place
3.7.3. Disassemble the bioreactor and clean all parts
3.7.4. Clean the porous sparger tip (optional)

4. References

4.1. ez-Control Hardware Manual for Autoclavable Bioreactors, Applikon Biotechnology, Hardware version 2, Documentation version 1.10.
4.2. ez-Control Operator Manual for Autoclavable Bioreactors, Applikon Biotechnology, Software version 1.6X, Documentation version 1.0.
4.3. ez-Control Software Reference Manual, ez-Control for Autoclavable and Single Use Applications, Applikon Biotechnology, Software version 1.6X, Documentation version 1.0.
4.4. BioXpert Lite Data Acquisition Program User Manual, Applikon Biotechnology, Software version 1.1X, February 2010.
4.5. Tuttnauer 3850 ELV Autoclave Operation, document number 14.02.01, effective date November 25, 2013
4.6. Labconco Purifier Class 2 Biosafety Cabinet SOP

5. Precautions

5.1. Alkaline solutions used for controlling pH are caustic. Read the Material Safety Data Sheet (MSDS) for hazards, handling and storage information. Wear personal protection equipment (PPE).
5.2. Do not put the stirrer motor or the heating blanket in the autoclave.

6. Responsibilities

6.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
6.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

7. Equipment and Materials

7.1. Applikon 3-liter glass autoclavable bioreactor:
7.1.1. Vessel
7.1.2. Head plate
7.1.3. Gas sparger (pre-mounted on the head plate)
7.1.4. Impeller (pre-mounted on the head plate)
7.1.5. Air outlet condenser (pre-mounted on the head plate)
7.1.6. Septum (pre-mounted on the head plate)

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7.1.7. Sample pipe (pre-mounted on the head plate)
7.1.8. pH sensor
7.1.9. DO sensor
7.1.10. Temperature sensor
7.1.11. Stirrer motor
7.1.12. Heating blanket
7.2. Applikon ez-Control bioreactor controller
7.3. Laboratory gasses:
7.3.1. Air compressor
7.3.2. O_2 (optional)
7.3.3. CO_2
7.4. pH 4.0 and pH 7.0 buffer standards
7.5. Liquid addition bottles
7.6. Alkaline solution (generally 1M sodium bicarbonate)
7.7. Gas filters, 0.2 µm
7.8. Autoclavable silicone tubing, size 14 (1.6 mm interior diameter)
7.9. Autoclavable silicone tubing, size 16 (3.1 mm interior diameter)
7.10. Autoclavable silicone tubing, size 25 (4.8 mm interior diameter)
7.11. Tubing clamps
7.12. Cotton and aluminum foil (for autoclaving)
7.13. Autoclave indicator tape
7.14. Culture medium
7.15. Culture inoculum
7.16. Autoclave (such as a Tuttnauer 3850 ELV)
7.17. Computer system with BioXpert Lite installed

8. Procedure

8.1. ***Preparing the controller***

8.1.1. Power up the controller and login

The controller is used to measure and control process variables (temperature, pH, dissolved oxygen, and stirrer speed) within a bioreactor. After switching on the power of the controller, it presents itself (after initialization) on the touch screen display with its Home screen, which is generally configured as the Synoptic View shown in Figure 2. The operator uses the touch screen display to monitor the bioreactor conditions and to enter process control parameters. Four authorization (login) levels allow access to various controller capabilities: View (initial level), Operator, System engineer, and Service engineer level. The View authorization level only allows process values to be monitored. To set process control parameters, the user needs to login as Operator. For an example of how to navigate the controller screens, see Example 1.

8.1.1.1. Power up the controller using the green power switch located on the back of the controller (upper right).
8.1.1.2. The touch screen display located on the front shows that the controller is initializing. Once initialization completes, the display switches to the Home screen.

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8.1.1.3. Login as Operator using the touch screen display:
8.1.1.3.1. Home > login button (top middle) > Operator > Login
8.1.1.3.2. Enter the Operator password: 0000
8.1.1.4. To logout:
Home > login button (top middle) > View
Note: logout is automatic after a period of inactivity (generally 10 minutes).
8.1.1.5. The touch screen backlight is switched off automatically after a period of inactivity (generally 30 minutes) and the touch screen goes dark. To switch on the backlight, touch the screen.

8.1.2. Enter the project name *(optional)*

The project name is displayed on the top of all controller screens in order to identify the bioreactor system and cell culture being cultivated.

8.1.2.1. Login as Operator per section 8.1.1.3 if not already logged in.
8.1.2.2. Home > project name button (top left)
8.1.2.3. Enter a descriptive name

8.1.3. Emergency stop and resume *(emergency only)*

The Emergency Stop button on the front of the control shuts down all control loops immediately and displays the Emergency Stop screen. Measurements continue to be collected.

8.1.3.1. In the event of an emergency requiring the bioreactor process to stop immediately, press the red Emergency Stop button located on the front of the controller.
8.1.3.2. To resume the bioreactor process:
8.1.3.2.1. Remove the cause of the Emergency Stop.
8.1.3.2.2. Turn the Emergency Stop button clockwise until it resets itself to the normal position.
8.1.3.2.3. Login as System Engineer.
8.1.3.2.4. Verify that the bioreactor is in a safe condition.
8.1.3.2.5. Select Resume on the Emergency Stop screen to restart the control loops.

8.2. ***Preparing the bioreactor***

8.2.1. Fill the bioreactor with culture medium

The bioreactor is filled with culture medium before it is autoclaved (if it will not damage the media) so that the medium is sterilized along with the bioreactor. Do not exceed the working volume of the bioreactor (2.4 liters for a 3-liter bioreactor). Leave enough space for inoculation and nutrients to be added during cultivation.

8.2.1.1. A process SOP should provide details regarding culture medium composition and volume.
8.2.1.2. Loosen the six mill nuts that fastens the head plate on the bioreactor vessel and remove the head plate.
8.2.1.3. Add culture medium to the vessel.
8.2.1.4. Mount the head plate on top of the vessel and fasten with the six mill nuts finger-tight.

8.2.2. ***Calibrate the pH sensor***

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Before the bioreactor is autoclaved, the pH (acidity) sensor needs to be calibrated with pH 4.0 and pH 7.0 buffer standards in order to obtain accurate measurement values during cultivation.

8.2.2.1. Measure the temperature of the pH buffer standards using a thermometer.

8.2.2.2. Remove the protective cap from the bottom of the pH sensor. Rinse the pH sensor with de-ionized water and pat it dry with a clean lint-free laboratory wipe.

8.2.2.3. Remove the pH sensor screw cap. Connect the pH sensor to the pH sensor cable on the right side of the controller. Verify that the pH sensor cable is plugged into the controller correctly.

8.2.2.4. Login as Operator per section 8.1.1.3 if not already logged in.

8.2.2.5. Go to the controller pH Settings screen: Home > pH (bottom)

8.2.2.6. Verify that the pH control loop is off (i.e. the pH Process Value button is grey or yellow, not green). If it is on, touch the button Stop pH controller.

8.2.2.7. Touch the button Calibrate pH to go to the pH Calibration screen. The numerical data for Slope, Offset and any Sample correction are displayed.

8.2.2.8. Touch the button 2-point calibration.

8.2.2.9. Enter the temperature of the buffer solutions using the numeric keypad.

8.2.2.10. When prompted for the pH value of the first buffer solution, put the pH sensor in the pH 4.0 buffer standard and wait until the shown process value stabilizes (shown near the Cancel button). Enter the pH value using the numeric keypad.

8.2.2.11. Rinse the pH sensor and repeat using the pH 7.0 buffer standard. Again, wait for the shown process value to stabilize and enter the corresponding pH.

8.2.2.12. Return to the pH Calibration screen to verify the newly found calibration data (slope and offset).

8.2.3. Mount sensors to the bioreactor

pH and DO sensors are mounted to the bioreactor before it is autoclaved so that the sensors are sterilized along with the bioreactor. Some systems also use a foam / level sensor.

8.2.3.1. Disconnect the cable of the pH sensor.

8.2.3.2. Cover the pH sensor connector with the pH sensor screw cap. Verify that the rubber gasket is in place between the sensor connector and the cap.

8.2.3.3. Insert the pH sensor into its port in the head plate and fasten it. See Figure 6 for the location of the pH sensor port.

8.2.3.4. Remove the protective cap from the bottom of the DO sensor. Repeat steps 8.2.3.2 and 8.2.3.3 for the DO sensor.

8.2.4. Prepare the liquid addition bottles

Liquid addition bottles are used to add liquids to the bioreactor aseptically (see Figure 7). For example, alkaline solution is added to raise pH and inoculum is added to begin cultivation. Addition bottles are sterilized along with the bioreactor.

8.2.4.1. A process SOP should provide details regarding alkaline solution composition and volume.

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8.2.4.2. Fill a liquid addition bottle with alkaline solution, no more than 2/3 full so that it can be autoclaved. Cap the alkaline bottle with a two-port top.

8.2.4.3. Connect the air inlet on the alkaline bottle to a gas filter using a short length (approx. 7 cm) of size 25 tubing. Do not clamp.

8.2.4.4. Add 5 mL laboratory grade water to an addition bottle to be used for transferring inoculum. This will improve the heat transfer during sterilization in the autoclave. Cap the inoculum transfer bottle with a two-port top.

8.2.4.5. Connect the air inlet on the inoculum transfer bottle to a gas filter using a short length (approx. 7 cm) of size 25 tubing. Do not clamp.

8.2.4.6. Connect the liquid outlet on the inoculum transfer bottle to an autoclavable male connector using a long length (approx. 50 cm) of size 25 tubing. Secure the needle to the tubing using a clamp or tape. Leave the cap on the needle.

8.2.4.7. Cover the gas filters and autoclavable male connector loosely with aluminum foil.

8.2.5. Mount connections to the bioreactor

Gas filters and silicone tubing connections are mounted to the bioreactor before it is autoclaved so that they are sterilized along with the bioreactor. See section 7 for the silicone tubing size specifications.

8.2.5.1. Connect one of the medium inlet triplet nipples to a second triplet nipple using a short length (approx. 7 cm) of size 14 tubing. Connect a medium length (approx. 15 cm) of size 14 tubing to the third medium inlet triplet nipple. Clamp the tubing closed.

8.2.5.2. Connect the addition pipe to the liquid outlet on the alkaline bottle using an extra-long length (approx. 75 cm) of size 25 tubing. Clamp the tubing closed.

8.2.5.3. Connect a medium length (approx. 15 cm) of size 25 tubing to the sample pipe. Clamp the tubing closed.

8.2.5.4. Connect the sparger inlet to a gas filter using a short length (approx. 7 cm) of size 25 tubing. Do not clamp.

8.2.5.5. Connect the bottom condenser nipple on the middle condenser nipple using a medium length (approx. 15 cm) of size 25 tubing.

8.2.5.6. Connect the top condenser nipple to a gas filter using a long length (approx. 50 cm) of size 25 tubing. Do not clamp.

8.2.5.7. Insert a septum into its holder in the head plate and fasten it.

8.2.5.8. Close all open tubing ends with cotton plugs and cover with aluminum foil.

8.2.5.9. Verify that the gas filters are open to avoid pressure differences during autoclaving. Cover the gas filters loosely with aluminum foil.

8.2.6. Autoclave the bioreactor and liquid addition bottles

The assembled bioreactor is autoclaved before cultivation in order to create a sterile environment inside the bioreactor. Do not autoclave the heating blanket or the stirrer motor. Single use bioreactors are not autoclaved; they are sterilized by the manufacturer.

8.2.6.1. Apply autoclave indicator tape to the aluminum foil on the alkaline bottle and the inoculum transfer bottle.

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8.2.6.2. Place the assembled bioreactor, the alkaline bottle, and the inoculum transfer bottle in the autoclave without disconnecting the tubing.
8.2.6.3. Loosen the caps on the alkaline bottle and the inoculum transfer bottle.
8.2.6.4. Close the autoclave and run it on the liquid cycle per the autoclave SOP.
8.2.6.5. When the cycle completes, allow the autoclave to cool gradually. Do not open the autoclave until the temperature in the autoclave has dropped below 90°C. After reaching that temperature, open the autoclave to allow it to cool down until the contents can be unloaded safely.
8.2.6.6. Tighten the caps on the alkaline bottle and the inoculum transfer bottle.
8.2.6.7. Remove the assembled bioreactor and the alkaline bottle together and place beside them on the right side of the controller without disconnecting the tubing.
8.2.6.8. Remove the inoculum transfer bottle and place it in a biological safety cabinet.
8.2.6.9. Allow to cool to room temperature.
8.2.6.10. Perform a visual inspection to verify that the autoclave indicator tape changed color and that the bioreactor is dry.
8.2.6.11. Remove aluminum foil and cotton plugs from tubing ends and gas filters on the bioreactor and alkaline bottle. Leave the foil on the inoculum transfer bottle.

8.3. ***Connecting the bioreactor to the controller***

After the assembled bioreactor is autoclaved, it must be connected to the controller.

8.3.1. Verify that all control loops are switched off

8.3.1.1. Login as Operator per section 8.1.1.3 if not already logged in.
8.3.1.2. Verify that all control loops are switched off. The Process Value buttons (bottom of Home Screen) should be gray or yellow. If necessary, stop controllers:
Home > Menu (top right) > Start/Stop all controllers > Stop all controllers

8.3.2. Connect the sensors

Sensors are connected electrically to the controller.

8.3.2.1. Place the bioreactor and alkaline bottle on the right side of the controller if they are not already.
8.3.2.2. Remove the pH sensor screw cap. Connect the pH sensor to the pH sensor cable on the right side of the controller. Verify that the pH sensor cable is plugged into the controller correctly.
8.3.2.3. Remove the DO sensor screw cap. Connect the DO sensor to the DO sensor cable on the right side of the controller. Verify that the DO sensor cable is plugged into the controller correctly.
8.3.2.4. Insert the temperature sensor into the thermometer pocket. Verify that the temperature sensor cable is plugged into the controller correctly. Fill the thermometer pocket with MilliQ water in order to decrease the dead time of the sensor and make temperature control more accurate.

8.3.3. Connect the heating blanket

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An electric heating blanket is used for warming the bioreactor as needed. Some systems also use a thermo circulator and cooling water for cooling the bioreactor.

8.3.3.1. Wrap the heating blanket around the bioreactor vessel (around the glass and inside the support legs). Position the blanket so that the volume markings on the vessel are visible. Fasten the blanket in place using the Velcro ends of the blanket.

8.3.3.2. Verify that the heating blanket is plugged into the controller correctly.

8.3.4. Connect the aeration

8.3.5. *Laboratory gasses (air, O_2, and CO_2) are added by the controller to the bioreactor sparger in order to control DO and to lower pH as needed.*

8.3.5.1. Connect the aeration outlet of the controller to the gas filter on the bioreactor sparger inlet using size 16 tubing.

8.3.5.2. Turn on the air compressor and set its regulator to 30 psi.

8.3.5.3. Open the CO_2 tank and set its regulator to 30 psi.

8.3.6. Connect the stirrer motor

The stirrer motor and the impeller are used to control agitation of the cell culture. When connecting the stirrer motor, it is helpful for the stirrer to be on.

8.3.6.1. Login as Operator per section 8.1.1.3 if not already logged in.

8.3.6.2. Go to the Stirrer Settings screen: Home > Stirrer (bottom left)

8.3.6.3. Set a slow stirrer speed: touch the button Stirrer setpoint and enter 60.

8.3.6.4. Start the stirrer: touch the button Start Stirrer controller.

8.3.6.5. Position the stirrer motor vertically over the bioreactor head plate and slowly lower it into place. Verify that the impellor is turning.

8.3.6.6. Stop the stirrer: touch the button Start Stirrer controller.

8.3.7. Connect the alkaline bottle

A peristaltic pump is used to dispense measured amounts of alkaline solution from an addition bottle to the bioreactor. The silicone tubing that connects the alkaline bottle to the bioreactor needs to be threaded through the pump. See Figure 8.

8.3.7.1. Login as Operator per section 8.1.1.3 if not already logged in.

8.3.7.2. Locate the alkaline pump on the right front panel of the controller. Open the pump cover.

8.3.7.3. Locate the tubing that connects the alkaline bottle to the bioreactor. Bend the middle of the tubing into a U shape and hold in one hand. Clip the tubing U into the lower pump clamp.

8.3.7.4. Turn the pump on manually:
Home > Menu > Manual control> Alkaline pump: On

8.3.7.5. Ease the tubing into the pump as the pump rotors are turning. Use care to avoid pinching fingers.

8.3.7.6. Turn the pump off.

8.3.7.7. Clip the tubing into the upper pump clamp.

8.3.7.8. Close the pump cover.

8.3.7.9. Turn the pump on manually and watch the solution being drawn from the bottle into the tubing. When the solution reaches the bioreactor, turn the pump off.

SOP: Applikon ez-Control Bioreactor Controller Operation

8.3.8. Enter process parameter settings

The pH, temperature, DO, and stirrer control loops should be adjusted to desired process parameter settings. Each control loop has settings for upper alarm limit, lower alarm limit, and setpoint. Additional settings for PID controls may also be set at this time.

8.3.8.1. A process SOP should provide details regarding process setpoints and alarm limits.

8.3.8.2. Login as Operator per section 8.1.1.3 if not already logged in.

8.3.8.3. Go to the pH Settings screen: Home > pH (bottom)

8.3.8.4. Enter the process settings for the pH control loop:

8.3.8.4.1. Enter the pH upper alarm limit:
pH settings > Alarm limits > Upper alarm limit
Enter the pH upper alarm limit value provided by the process SOP.

8.3.8.4.2. Enter the pH lower alarm limit:
pH settings > Alarm limits > Lower alarm limit
Enter the pH lower alarm limit value provided by the process SOP.

8.3.8.4.3. Enter the pH setpoint:
pH settings > pH setpoint
Enter the pH setpoint value provided by the process SOP.

8.3.8.4.4. Enter any additional pH PID controls provided by the process SOP:
pH settings > pH controller setup

8.3.8.5. Repeat for each of the temperature, DO, and stirrer control loops.

8.3.9. Start all control loops

The DO sensor needs to be polarized for at least 6 hours and the temperature and pH need to be stabilized at setpoint before the DO sensor can be calibrated.

8.3.9.1. Login as Operator per section 8.1.1.3 if not already logged in.

8.3.9.2. Start all control loops:
Home > Menu > Start/Stop all controllers > Start all controllers

8.3.9.3. Go to the Home screen and verify that the control loops are on. The Process Value buttons (bottom of Home Screen) should change to green or red, the actuators (heat, pumps, valves, and stirrer) should begin activating, and the impeller should begin turning.

8.3.9.4. Allow the process to run for at least 6 hours.

8.3.10. Calibrate the DO sensor

The DO measurement is based on the polarographic principle (Clark-cell). Therefore, the sensor must be polarized for at least 6 hours before it can be calibrated.

8.3.10.1. Login as Operator per section 8.1.1.3 if not already logged in.

8.3.10.2. Verify that the medium in the bioreactor is stable at process temperature.

8.3.10.3. Stop the DO control loop:
Home > DO (bottom) > Stop DO controller

8.3.10.4. Go to the DO Calibration screen:
Home > DO > Calibrate DO
The numerical data for Slope and Offset are displayed.

SOP: Applikon ez-Control Bioreactor Controller Operation

8.3.10.5. Verify if the measuring range is set to Air. If not, set it for air:
Calibrate DO > Set measurement range > Measurement range for air

8.3.10.6. Open the aeration valve manually:
Home > DO > Manual control > O2 Valve: On

8.3.10.7. Continue aeration until DO reading is stable (15 to 20 minutes).

8.3.10.8. Close the aeration valve manually:
Home > DO > Manual control > O2 Valve: Off

8.3.10.9. Set the DO calibration value to 100%:
Home > DO > Calibrate DO > Calibrate > Enter Calibration Value > 100

8.3.10.10. Return to the DO Calibration screen to verify the newly found calibration data (slope and offset). The expected slope value of the sensor (for measurement range for air) is:
2.0 to 4.0 at 25 °C
1.5 to 3.0 at 37 °C

8.3.10.11. Start the DO control loop: DO (bottom) > Start DO controller.

8.3.11. Reset dose monitor values

When all control loops are at set-point, the bioreactor system is ready for cultivation (inoculation). All Dose Monitor values should be reset to 0 ml.

8.3.11.1. Go to the Home screen and verify that the control loops are on. Allow the process to run until all control loops are at set-point.

8.3.11.2. Reset all dose monitor values:
Home > Menu > Dose Monitor > Reset all dose monitors > Are you sure? Yes

8.4. Starting the cultivation

8.4.1. Inoculate the bioreactor

Once the process parameters in the bioreactor are at their setpoints, the inoculum is added to the bioreactor aseptically. This SOP makes use of a sterile addition bottle for this purpose.

8.4.1.1. Fill the sterile inoculum transfer bottle with inoculum using aseptic technique per the biosafety cabinet SOP. Do not remove the foil from the gas filter or the autoclavable male connector. Recap the bottle before removing it from the biosafety cabinet.

8.4.1.2. Login as Operator per section 8.1.1.3 if not already logged in.

8.4.1.3. Stop all control loops:
Home > Menu > Start/Stop all controllers > Stop all controllers

8.4.1.4. Disinfect the septum with 70% ethanol.

8.4.1.5. Remove the foil and cap from the needle, sterilize the needle with a flame, and pierce the needle through the septum.

8.4.1.6. Remove the foil from the gas filter on the inoculum transfer bottle. Transfer the inoculum from the bottle to the bioreactor using a large volume syringe to pump air through the gas filter into the bottle.

8.4.1.7. Remove the needle from the septum. Disconnect the needle from the tubing and dispose in a sharps container.

8.4.1.8. Re-start all control loops:
Home > Menu > Start/Stop all controllers > Start all controllers

SOP: Applikon ez-Control Bioreactor Controller Operation

8.5. ***Monitoring the cell culture***

8.5.1. View process data

Process values for the last 72 hours are stored in controller memory and can be displayed using the Trend View screen. Process values for the entire run are stored and displayed using BioXpert Lite.

8.5.1.1. To view process data for a specific process parameter using the Trend View:

8.5.1.1.1. Login as Operator per section 8.1.1.3 if not already logged in.

8.5.1.1.2. Home > process value button (2nd row from the bottom)

8.5.1.1.3. Touch the X-axis button (bottom left) in order to change the range of the time axis (between 1 and 72 hours).

8.5.1.1.4. Touch the Y-axis upper limit button (top right) or lower limit button (bottom right) in order to change the range of the process value axis.

8.5.2. Sample the cell culture

Periodically, the cell culture is sampled in order to measure cell concentration and perform product assays per the process SOP. Some bioreactors include a sample system connected to the sample pipe which enables cell culture to be drawn into a sample bottle that can then be replaced aseptically. This system simply uses a pipette to draw cell culture from the sample pipe.

8.5.2.1. Locate the clamped tubing connected to the sample pipe on the bioreactor head plate. Connect a pipette to the sample pipe tubing.

8.5.2.2. Release the clamp, withdraw 5 mL of cell culture, and re-clamp. (Sample pipe volume = 4mm ID X 25 cm = 3.2 cm^3 = 3.2 mL) Discard this sample.

8.5.2.3. Again, release the clamp, withdraw 5 mL of cell culture, and re-clamp. Use this sample for cell counts and product assays per the process SOP.

8.6. ***Harvesting the cell culture***

A process SOP should specify when the cell culture is to be harvested.

8.6.1. Stop the controllers

8.6.1.1. Login as Operator per section 8.1.1.3 if not already logged in.

8.6.1.2. Stop all control loops:
Home > Menu > Start/Stop all controllers > Stop all controllers

8.6.1.3. Close the CO_2 tank.

8.6.1.4. Turn off the air compressor.

8.6.2. Disconnect the bioreactor

The bioreactor must be disconnected from the controller so that the bioreactor can be decanted.

8.6.2.1. Locate the tubing that connects the alkaline bottle to the bioreactor. Clamp the tubing near the bioreactor. Disconnect the tubing from the alkaline bottle and remove the tubing from the controller pump.

8.6.2.2. Lift the stirrer motor from the bioreactor head plate and set the motor aside.

8.6.2.3. Disconnect the gas filter on the bioreactor sparger inlet from the tubing to the aeration outlet of the controller.

8.6.2.4. Unwrap the heating blanket from around the bioreactor vessel and set the blanket aside being sure it is lying flat.

SOP: Applikon ez-Control Bioreactor Controller Operation

8.6.2.5. Disconnect the pH sensor cable from the pH sensor. Cover the pH sensor connector with the pH sensor screw cap.

8.6.2.6. Repeat step 8.6.2.5 for the DO sensor.

8.7. ***Cleaning the bioreactor***

8.7.1. Clean and store the sensors

8.7.1.1. Remove pH and DO sensors from the bioreactor head plate.

8.7.1.2. Rinse the pH and DO sensors thoroughly with MilliQ water, being careful to remove all broth-residue. Gently pat dry with a clean lint-free laboratory wipe. Spray with 70% IPA and gently pat dry with a clean lint-free laboratory wipe

8.7.1.3.. Rinse with MilliQ water and pat dry with a clean lint-free laboratory wipe.

8.7.1.4. Fill the protective cap of the pH sensor 1/2 full with 3M potassium chloride (KCl) solution. Cover the tip of the pH sensor with its protective cap. Verify that the pH electrode is completely immersed in KCl solution.

8.7.1.5. Cover the tip of the DO sensor with its protective cap. The DO sensor may be stored dry.

8.7.1.6. Cover the pH sensor connector with the pH sensor screw cap. Repeat for the DO sensor.

8.7.2. Decant the cell culture

8.7.2.1. Place the disconnected bioreactor in a biosafety cabinet if specified by the process SOP.

8.7.2.2. Loosen the six mill nuts that fasten the head plate on the bioreactor vessel and remove the head plate.

8.7.2.3. Decant the cell culture into a suitable container per the process SOP.

8.7.2.4. Re-mount the head plate on top of the vessel and fasten with the six mill nuts finger-tight.

8.7.3. Pre-clean the bioreactor in place

8.7.3.1. Fill the bioreactor with a working volume of 0.1M NaOH solution (2.4 liters for a 3-liter bioreactor).

8.7.3.2. Connect the stirrer motor per section 8.3.6.

8.7.3.3. Activate the stirrer at 250 RPM for 30 minutes. Visual check for dissolution of foam, debris and other contamination in the bioreactor.

8.7.3.4. Stop the stirrer. Lift the stirrer motor from the bioreactor head plate and set the motor aside.

8.7.3.5. Drain the bioreactor.

8.7.4. Disassemble the bioreactor and clean all parts

8.7.4.1. Remove all tubing and gas filters from the bioreactor head plate assembly and discard in biohazardous waste.

8.7.4.2. Remove the septum from the head plate.

8.7.4.3. Remove the air outlet condenser from the head plate and disassemble the condenser for cleaning.

8.7.4.4. Remove the head plate from the bioreactor vessel.

8.7.4.5. Clean all parts carefully and thoroughly using a small soft bristle brush (e.g. tooth brush) and a dilute laboratory glassware cleaner. Rinse with MilliQ water and then repeat with a 10% bleach solution. Rinse thoroughly with MilliQ

SOP: Applikon ez-Control Bioreactor Controller Operation

water and spray with 70% IPA and place on paper towels on a lab bench to dry.

8.7.4.6. Let dry all the parts.

8.7.5. Clean the porous sparger tip (optional)

Depending on the type of medium that is used (presence of proteins and/or peptides), the porous sparger tip may require a special cleaning procedure.

8.7.5.1.1. Remove the sparger tip from the air inlet pipe.

8.7.5.2.2. Soak the sparger overnight in a solution of 10 mg/mL pepsin / 0.01M HCl.

8.7.5.3.3. Use ultrasonic cleaning with water and/or ethanol.

8.7.5.4.4. Replace the sparger tip onto the air inlet pipe.

SOP: Applikon ez-Control Bioreactor Controller Operation

9. Attachments

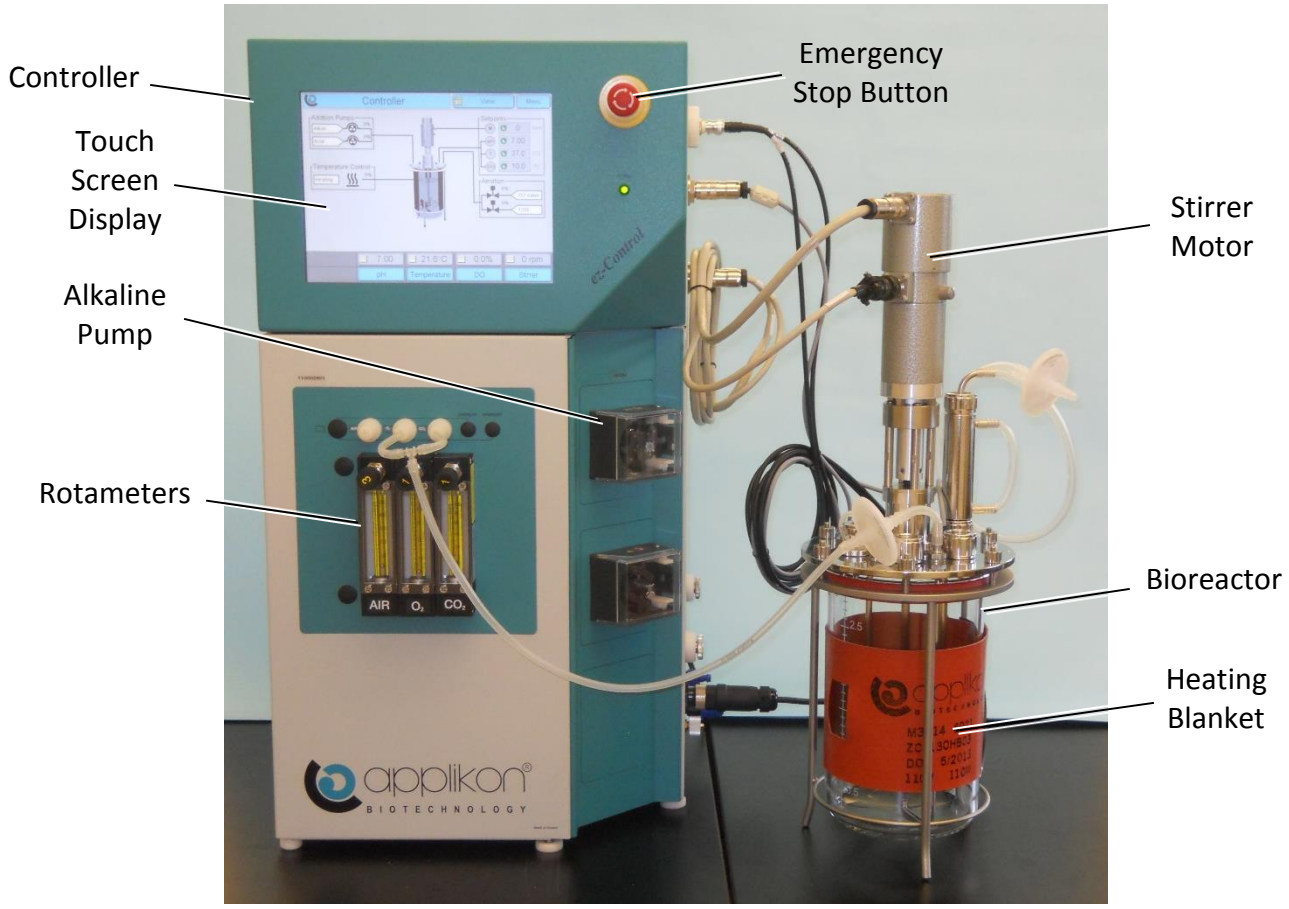

Figure 1. Applikon ez-Control Bioreactor Controller and Bioreactor

SOP: Applikon ez-Control Bioreactor Controller Operation

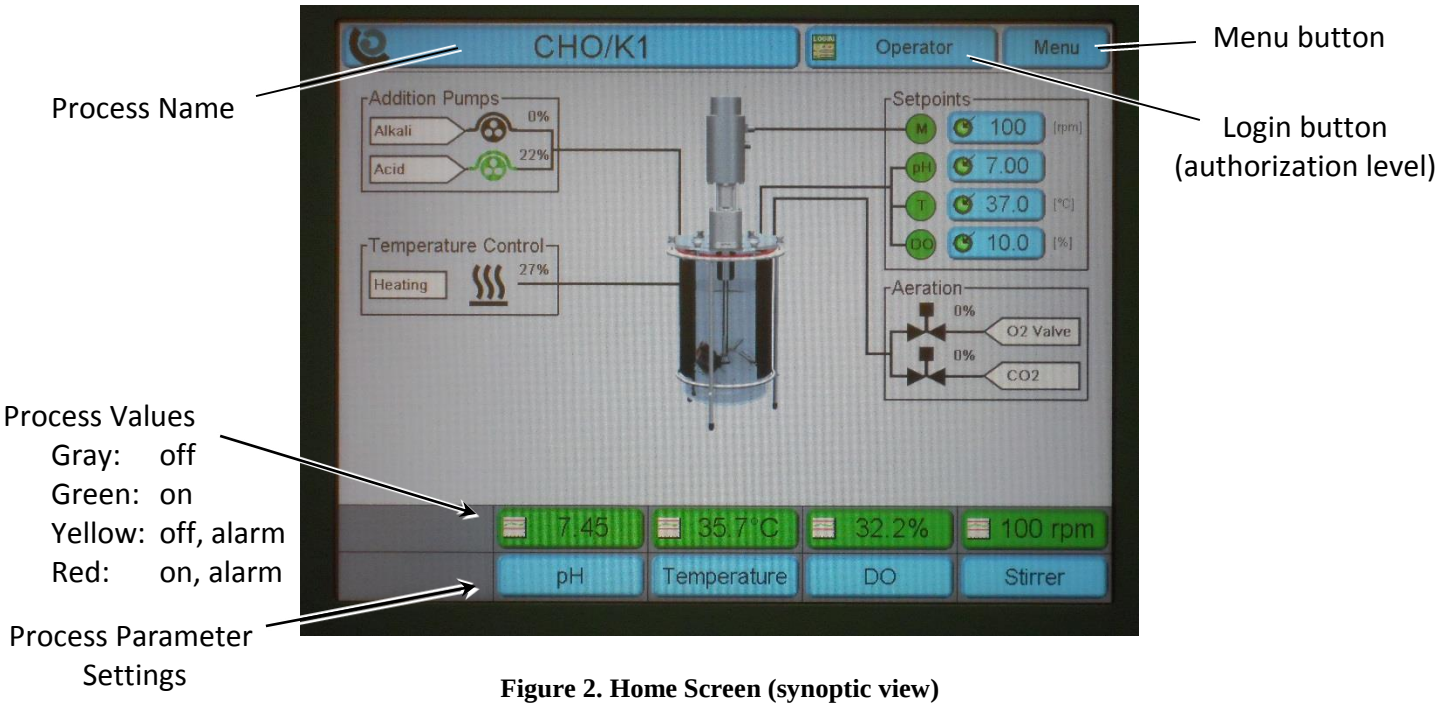

Figure 2. Home Screen (synoptic view)

Example 1. Navigating the Controller Screens to Login

Instructions	Explanation
Home > Login (top middle) > Operator > Login Enter the Operator password: "0000"	On the Home screen, touch the Login button (top middle). The display changes to the Access Control screen. On the Access Control screen, touch the button Operator (left side). The display changes to the Operator screen. On the Operator screen, touch the button Login. The display changes to a keyboard and prompts for the Operator password. Enter the Operator password: "0000" Then touch the button Enter. The display changes back to the Home screen.

SOP: Applikon ez-Control Bioreactor Controller Operation

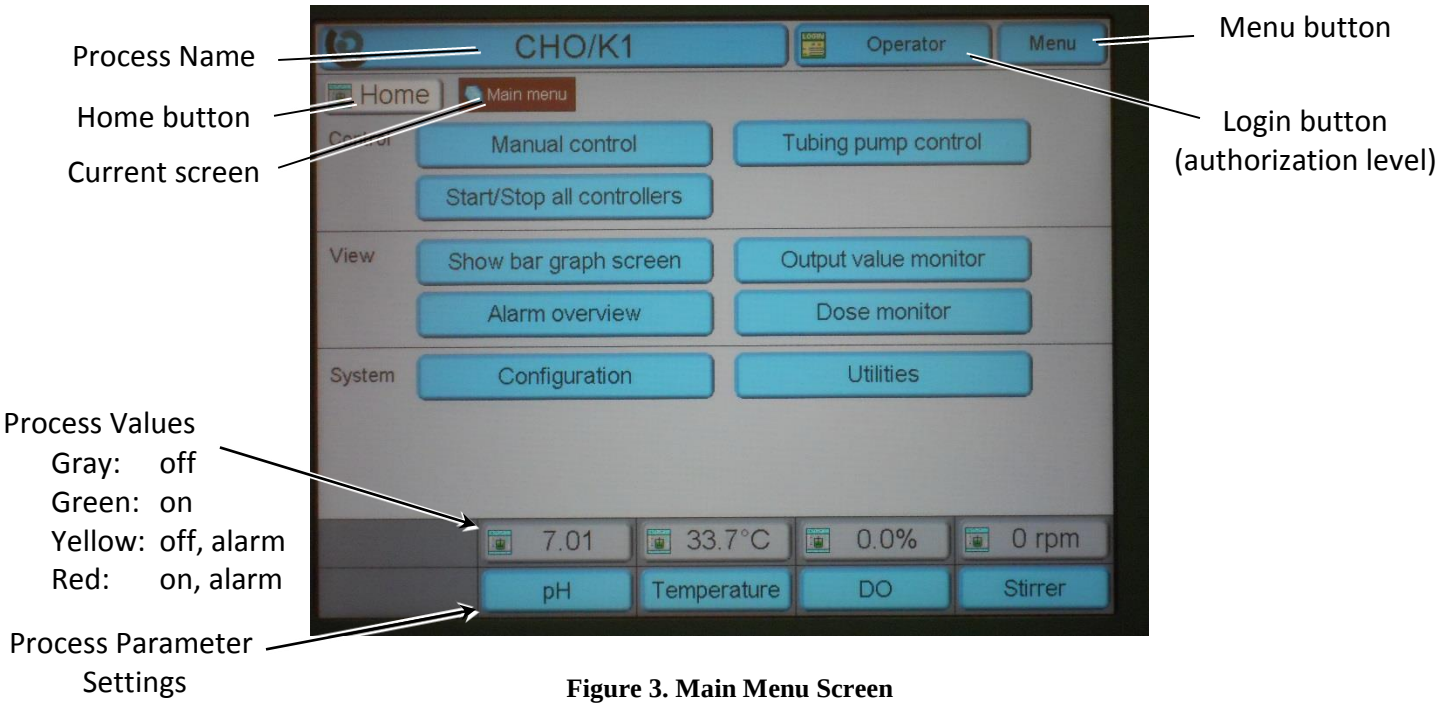

Figure 3. Main Menu Screen

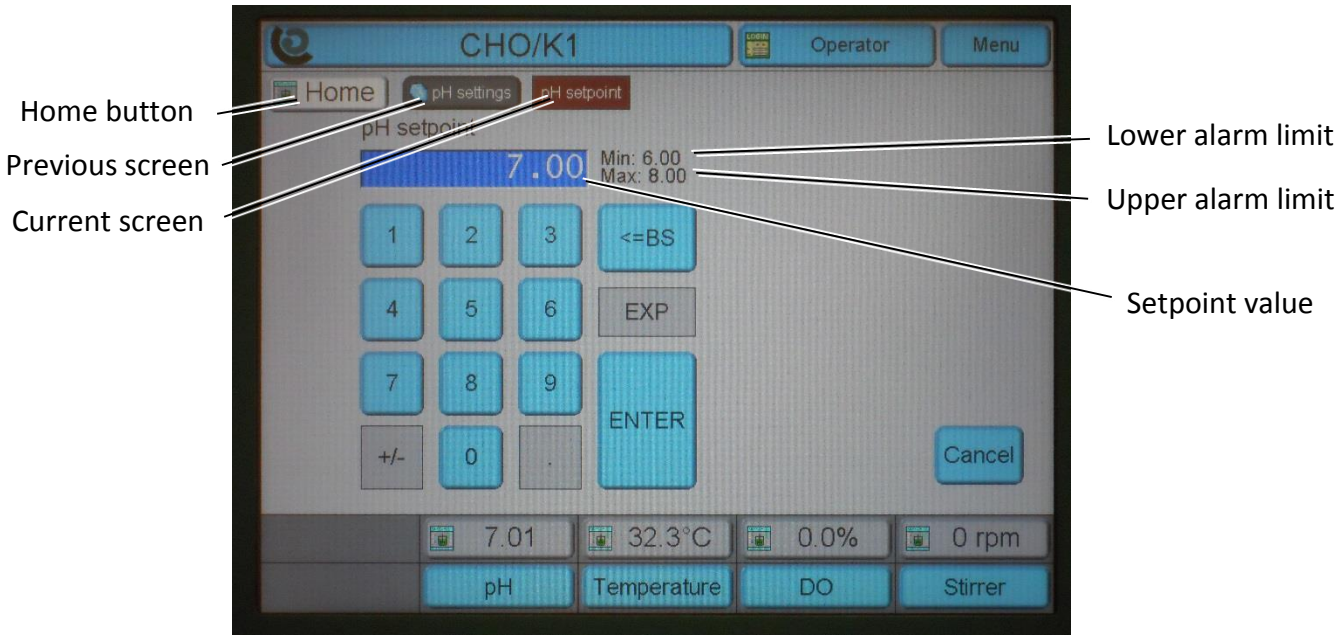

Figure 4. pH Setpoint Screen

SOP: Applikon ez-Control Bioreactor Controller Operation

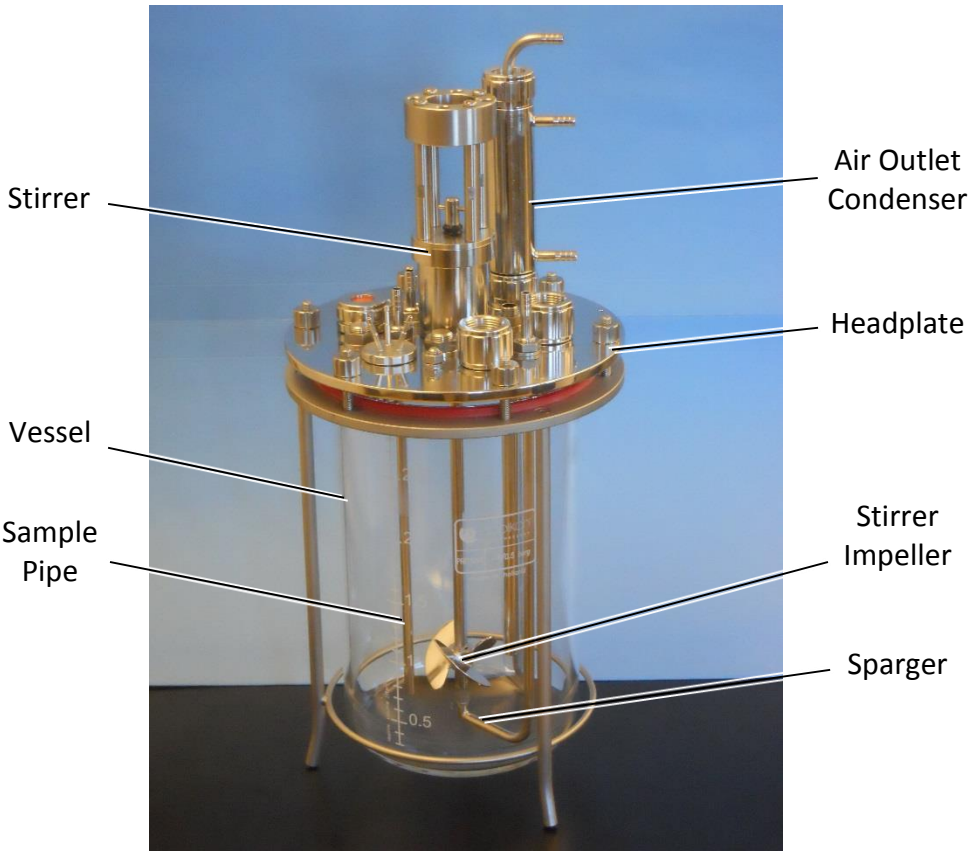

Figure 5. Applikon 3-Liter Glass Autoclavable Bioreactor

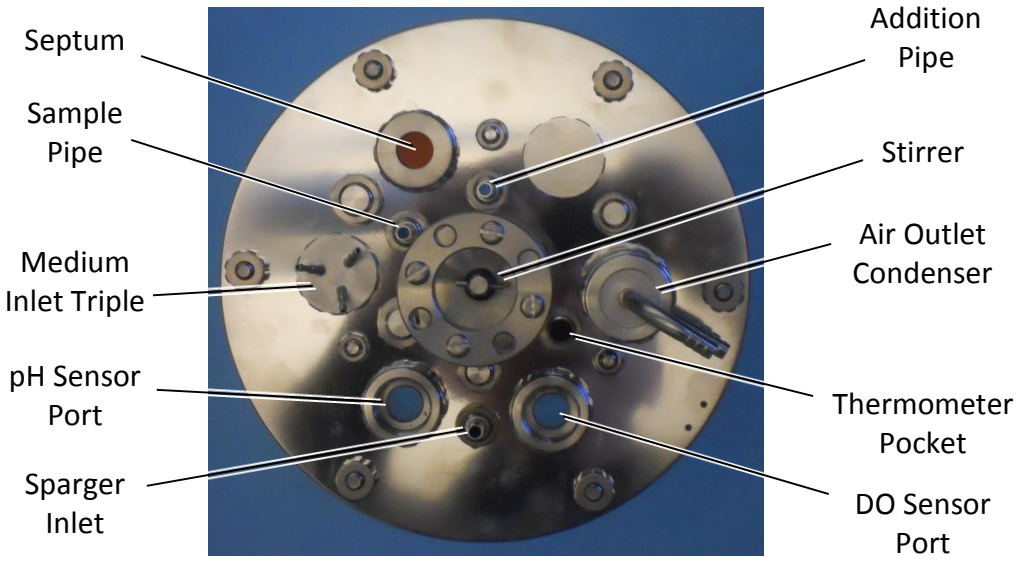

Figure 6. Bioreactor Head Plate (top view, unassembled)

SOP: Applikon ez-Control Bioreactor Controller Operation

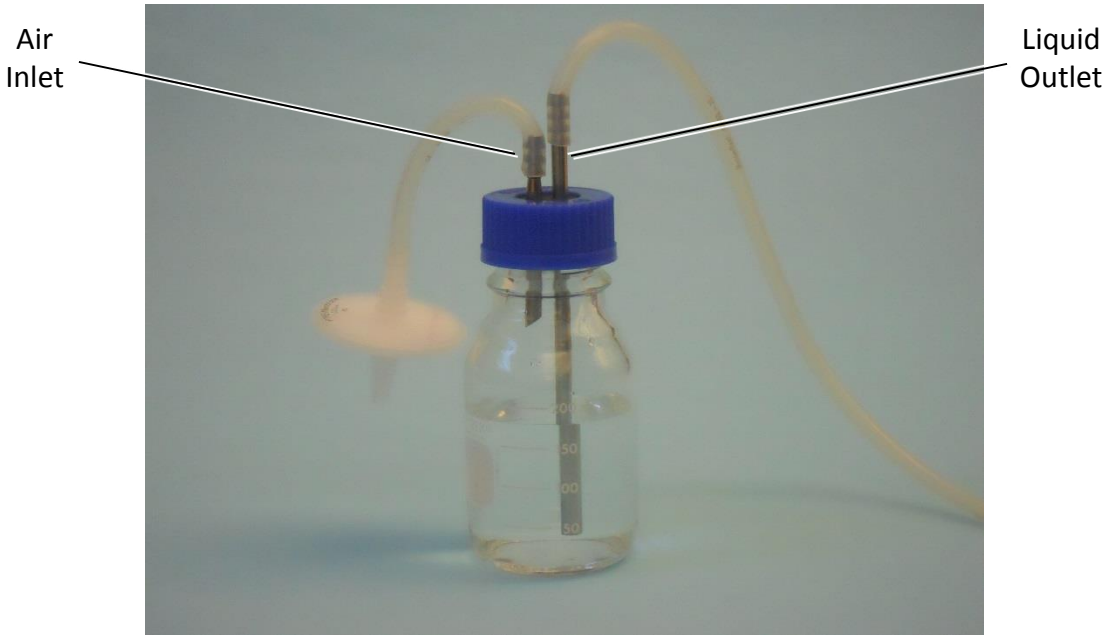

Figure 7. Liquid Addition Bottle

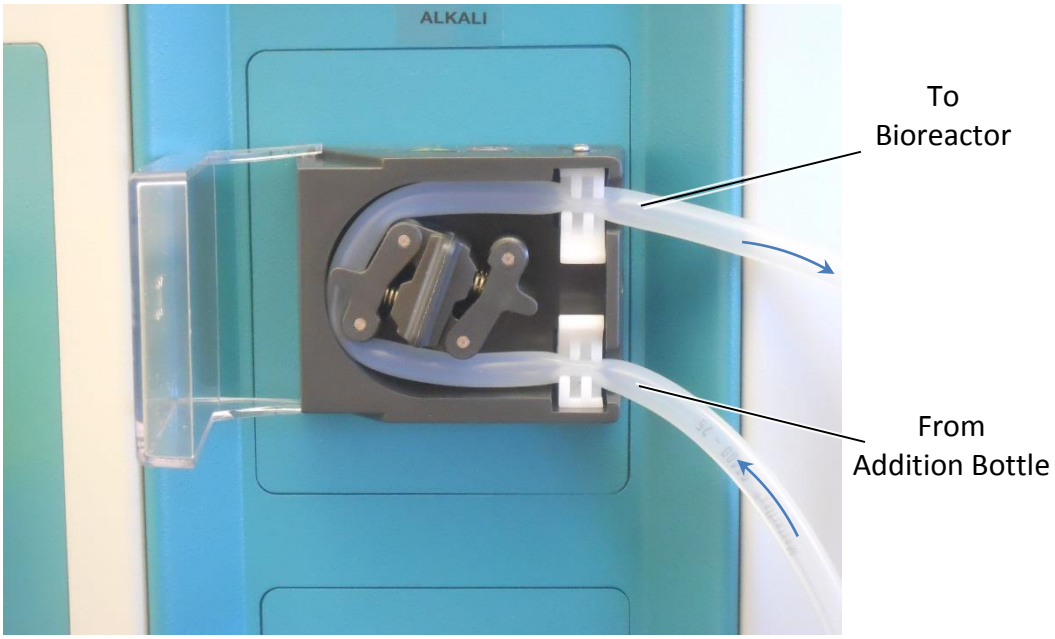

Figure 8. Peristaltic Pump (Alkaline Pump)

SOP: Applikon ez-Control Bioreactor Controller Operation

10. History

Revision Number	***Effective Date***	***Preparer***	***Description of Change***
0	20DEC13	John Buford	Initial release
1	16SEP14	Jason McMillan	Various fixes and adjustments

SOP: Operation of Life Technologies Countess II Cell Counter

Approvals

Preparer: Jason McMillan	Date: 09JUL15
Reviewer: Dr. Maggie Bryans	Date: 09JUL15
Reviewer: Hetal Doshi	Date: 10JUL15

1. **Purpose**
   1.1. To perform cell count and viability measurements of trypan blue stained samples.
2. **Scope and Applicability**
   2.1. Applies to the counting and viability measurement of trypan blue stained samples using the Life Technologies Countess II Cell Counter.
3. **Summary of Method**
   3.1. Turn on the Countess II Automated Cell Counter.
   3.2. Mix cell aliquot with trypan blue stain.
   3.3. Load 10µl of cell/trypan blue stain mixture into Side A of the Countess Cell Counting Chamber Slide.
   3.4. Insert the Countess Cell Counting Chamber Slide into the slide port.
   3.5. Perform count.
   3.6. Remove the Countess Cell Counting Chamber Slide and turn around the slide, and reinsert Side B into the slide port.
   3.7. Perform count.
   3.8. Remove the Countess Cell Counting Chamber Slide and dispose of into the sharps container.
   3.9. Load USB drive into the Countess II Automated Cell Counter and save data.
   3.10. Turn off the Countess II Automated Cell Counter.
4. **References**
   4.1. Countess II/Countess II FL Automated Cell Counter Quick Reference Guide
5. **Definitions**
   5.1. N/A
6. **Precautions**
   6.1. N/A
7. **Responsibilities**
   7.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   7.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
8. **Equipment and Materials**
   8.1. Life technologies Countess II Automated Cell Counter
   8.2. Countess Cell Counting Chamber Slide
   8.3. 0.4% trypan blue stain
   8.4. P20 micropipette

SOP: Operation of Life Technologies Countess II Cell Counter

8.5. P20 micropipette tips
8.6. Microcentrifuge tube
8.7. Microcentrifuge rube rack

9. Procedure

9.1. Turn on the Countess II Automated Cell Counter by pressing the switch on the lower, right hand corner on the back of the system.
  9.1.1. The power indicator light will illuminate and the 7" capacitive touchscreen will illuminate and flash the Life Technologies logo, Countess II Automated Cell Counter logo, Life technologies and Countess II Automated Cell Counter logos, and finally settling on the home screen that prompts the user to "Insert Slide."
9.2. Prepare sample by adding 10µl of cell suspension to 10µl of 0.4% trypan blue stain.
9.3. Remove a Countess Cell Counting Chamber Slide and open by tearing the wrapper at the portion labeled "Tear Here."
  9.3.1. Remove the Countess Cell Counting Slide and locate Side A.
9.4. Load 10µl of the sample mixture into Side A of the Countess Cell Counting Chamber Slide.
9.5. Insert the Countess Cell Counting Slide, Side A first, into the slide port until you hear a soft click.
9.6. The instrument automatically illuminates the sample and focusses the cells.
  9.6.1. To enable the auto focus to function properly, manually focus the cells using the "Focus" slider and press "Set" to set the optimal focus.
  9.6.2. Set exposure using the "Light source" slider, and press "Capture" on the 7" capacitive touchscreen. The instrument captures the image and displays the results (total concentration, percentage and concentration of live and dead cells).
9.7. To count the second chamber, remove the Countess Cell Counting Slide and turn around the slide to Side B, and reinsert into the slide port until you hear a soft click.
  9.7.1. Repeat steps 9.5. and 9.6.
9.8. To save data
  9.8.1. On the 7" capacitive touchscreen press "Save"
  9.8.2. Insert USB Drive into the USB port
  9.8.3. Press "Save" on the 7" capacitive touchscreen, a check mark with the phrase "Save Complete" will appear.
  9.8.4. On the 7" capacitive touchscreen press "Close."
  9.8.5. Remove USB drive
9.9. Remove the Countess Cell Counting Slide and dispose of in a sharps container.
9.10. Turn off the Countess II Automated Cell Counter by pressing the switch on the lower, right side on the rear of the instrument.

10. Attachments

SOP: Operation of Life Technologies Countess II Cell Counter

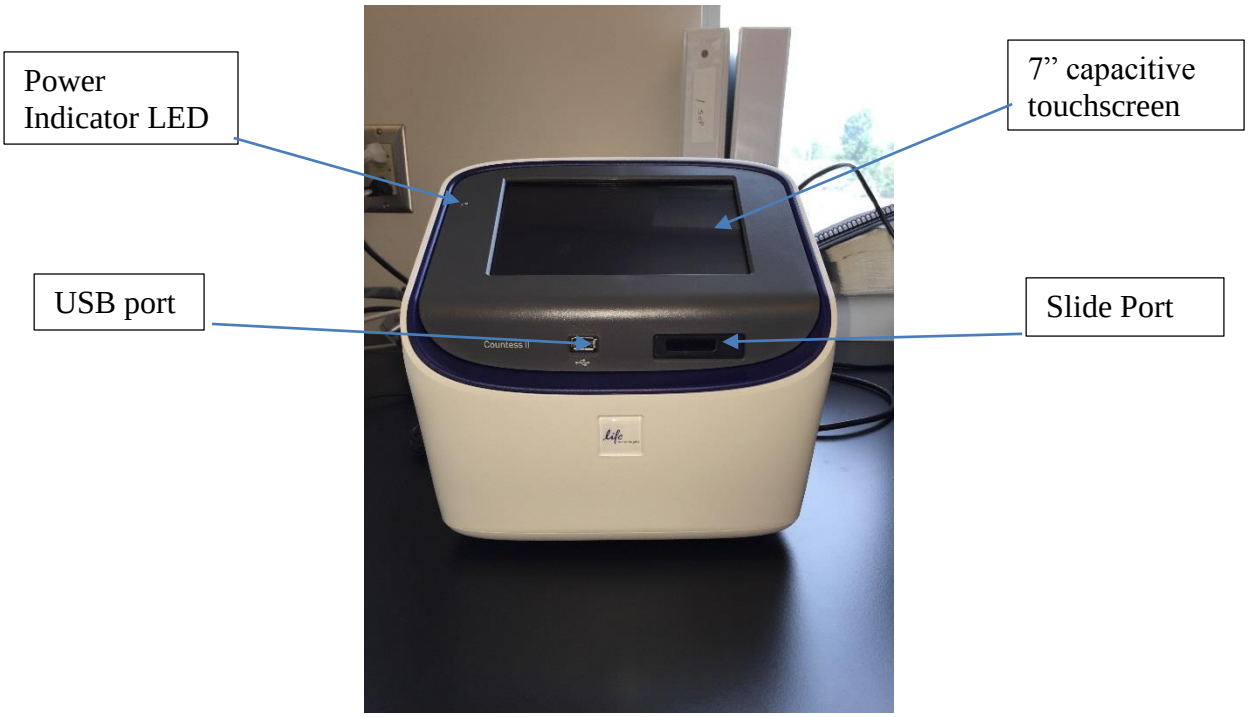

Figure 1. Countess II Cell Counter front panel

11. History

Revision Number	***Effective Date***	***Preparer***	***Description of Change***
0	09JUL15	Jason McMillan	Initial release

SOP: Trypan Blue Assay

Approvals:

Preparer: Dr. Maggie Bryans Date: 02JAN14
Reviewer: Jason McMillan Date: 02JAN14

1. **Purpose:**
   1.1. Use of the Trypan Blue Assay.
2. **Scope:**
   2.1. Applies to determining viable cell count of mammalian and insect cells.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. http://plaza.ufl.edu/johnaris/Protocols/MiscMethods/HemoCytometer.pdf
   4.2. microscope SOP
5. **Definitions:**
   5.1. Hemacytometer: a specialized microscope slide with etched glass in grid formation
6. **Precautions:**
   6.1. Trypan Blue Solution is a teratogen. It may cause birth defects. It may cause cancer. Wear gloves, eye protection and a lab coat.
7. **Materials:**
   7.1. 0.4% Trypan Blue Solution
   7.2. microfuge tubes
   7.3. P-20 micropipette and tips
   7.4. cell sample in solution
   7.5. hemacytometer
   7.6. hemacytometer coverslip
   7.7. microscope
   7.8. lab towels
   7.9. lab tissues such as Kimwipes
   7.10. deionized water
   7.11. push button counter
8. **Procedure:**
   8.1. **Mix Trypan Blue Solution with cell sample solution**
      8.1.1. Mix culture sample well to resuspend cells.
      8.1.2. Remove 20µL of culture sample and dispense into a microfuge tube.
      8.1.3. Add 20µLof 0.4% Trypan Blue Solution to the same tube.
      8.1.4. Mix the above solution by gently aspirating and dispensing the solution with the micropipette. Proceed to the next step immediately.
   8.2. **Transfer sample to hemacytometer**
      8.2.1. Center the coverslip on top of the hemacytometer. The metal notches should be partially exposed.

SOP: Trypan Blue Assay

8.2.2. Hold the micropipette straight up and dispense 10µL of the cell/Trypan Blue solution into a notch of the hemacytometer. The tip of the pipette should be very close to the metal surface. The solution will spread through capillary action.

8.3. **Observe cells under the microscope**

8.3.1. Turn on the microscope per SOP.

8.3.2. Place the hemacytometer on the microscope stage.

8.3.3. Focus on the hemacytometer grid using 100X magnification (10X objective lens). Live cells are clear. Dead cells are blue.

8.4. **Count cells**

8.4.1. The grid is divided into four main quadrants (Figure 2). Beginning with quadrant 1 and moving through to quadrant 4, depress the correct button on the push button counter for every cell in each square.

8.4.1.1. Make sure the counter is set to 0. Count total live cells first. After live cell count is completed, count total dead cells.

8.4.1.2. Count in a serpentine fashion: work left to right across the top row of the quadrant. Move down to the second row and count the cells in each square moving right to left. Change to opposite direction each time a row is completed.

8.4.1.3. Count cells touching the top and left borders of a main quadrant, but not the bottom and right borders. **Do not count cells outside of the main quadrants**.

8.4.1.4. Record the number of live and dead cells each time a quadrant is completed.

8.4.2. Repeat counting procedure per section 8.4.1. for the second grid.

8.4.3. Average the cell counts of the two grids to obtain the live cell count for the viable cell concentration calculations.

8.5. **Clean the hemacytometer**

8.5.1. Remove the coverslip.

8.5.2. Blot dry the coverslip and hemacytometer on a lab towel.

8.5.3. Rinse the cover slip and hemacytometer with 70% EtOH by holding each one over a lab towel and using a squirt bottle of DI water. **Note:** Handle the hemacytometer and coverslip gently. The coverslip is not disposable. Do not discard it.

8.5.4. Dry the coverslip and hemacytometer with a lab tissue.

8.6. **Calculate viable cell concentration.**

8.6.1. Formula to determine live cell count: **C = (N/V) x D**

C = live cell count in cells per milliliter

N = total number of live cells obtained from averaging the number of live cells counted in the two grids

V = volume of counting area

Note: The total volume of the four quadrants is 0.0004mL. (Each quadrant is 0.0001mL.)

D = dilution factor. For this procedure the dilution factor is 2.

8.7. Calculate percent viability

SOP: Trypan Blue Assay

8.7.1. Formula for percent viability: **% viability = (live cell count/total cell count)*100**

9. Attachments:

9.1. Figure 1: Diagram of hemacytometer and cover glass

9.2. Figure 2: Diagram of hemacytometer quadrants

10. History:

Name	Date	Amendment
Kari Britt	26JUL07	Initial release
Deb Audino	04APR08	College name change, format of history
Kari Britt	05AUG10	Proofreading, formatting and grammar edits throughout
Jason McMillan	02JAN14	College name change, fixed quadrant number labels

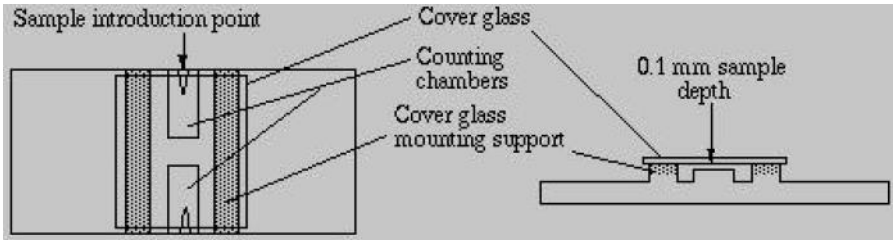

Figure 1: Diagram of hemacytometer and cover glass
Image: http://plaza.ufl.edu/johnaris/Protocols/MiscMethods/HemoCytometer.pdf

SOP: Trypan Blue Assay

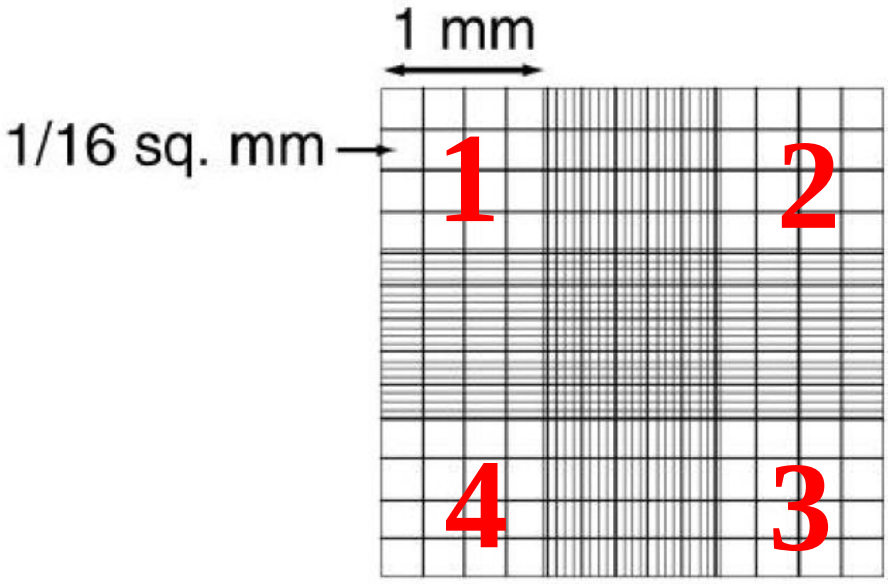

Figure 2: Diagram of hemacytometer quadrants

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

Record Keeping Standards:

For each step in the batch record: the operator of the task will enter their initials (each operator has their own unique set of initials) and the date in the appropriate section(s) of the batch record. Another operator must initial and date in the appropriate section of the batch record to verify that the task was completed per SOP. No operator will verify their own work at any point. "If you didn't document it, you didn't do it!"

Batch records will be completed in blue or black ball point pen ONLY, and must be legible.

Any errors on a batch record will be crossed out with a single line through the error with the initials of the operator and the date. Corrections will be written in next to the crossed out error.

Use the following format to record dates: DDMMMYY. For July 10, 2006 use 10JUL06.

Use the 24 hour clock or "military time" to record time: 3:00pm would be written as 15:00.

Any and all deviations from a protocol or SOP, including abnormal results or retests performed, will be entered into the comments section at the end of each batch record. Be as detailed and specific as possible, include all steps taken before and/or after an abnormal reading, and provide an explanation for any deviations from a step.

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

1. Initial Media Preparation		
Clean, assemble and autoclave two 100mL Bellco Spinner flasks per SOP. Spinner flask ID#_____Spinner flask ID#_____	Operator/Date	Verifier/Date
Obtain sterile Fetal Bovine Serum (FBS). Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____	Operator/Date	Verifier/Date
Obtain sterile Ham's F12 Medium Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____	Operator/Date	Verifier/Date
Sterilely add 21.6mL of Ham's F12 Medium to a spinner flask. Repeat with the second spinner flask 100mL spinner flask ID#_____Vol of Ham's F12_____mL 100mL spinner flask ID#_____Vol of Ham's F12_____mL	Operator/Date	Verifier/Date
Sterilely add 2.4mL ± 1 mL of FBS to each spinner flask. 100mL spinner flask ID#_____Vol of FBS_____mL 100mL spinner flask ID#_____Vol of FBS_____mL	Operator/Date	Verifier/Date
Label spinner flasks as 90% Ham's F12, 10% FBS, [date], [group#], [operator initials].	Operator/Date	Verifier/Date
Place spinner flasks containing CHO cell media in the CO_2 incubator. Set the speed of the magnetic stirrer to the maximum setting that ensures an even vortexing of the culture without foaming.	Operator/Date	Verifier/Date
Verify that CO_2 is set to 5±0.5% and that temperature is set to 37±0.5ºC. CO_2_____% Temperature _____ ºC	Operator/Date	Verifier/Date
Check media for contamination after a minimum of 24 hrs. Elapsed Incubation Time _____ 100mL spinner flask ID_____ Contamination: Y / N (Circle) 100mL spinner flask ID_____ Contamination: Y / N (Circle)	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

2. Inoculation of Spinner Flasks		
Pre-warm the spinner Flasks containing CHO Cell Culture Medium at 37° C ± 0.5°C overnight.	Operator/Date	Verifier/Date
Remove two vials of CHO cells from storage in the -86°C freezer. Vial ID:_____ _____ Vial ID:_____ _____	Operator/Date	Verifier/Date
Sterilely transfer the entire contents of each 1mL vial of thawed CHO Cells into the previously prepared Spinner Flask containing 100mL CHO Cell Culture Medium using a 2mL sterile pipette. **Swirl** to mix. Be sure to not add any CHO Cells to the Spinner Flask labeled "Blank."	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

3. Scale Up to 100ml Media Preparation		
Remove the **inoculated** 100mL Bellco Spinner flask from the CO_2 incubator. Spinner flask ID#_____Spinner flask ID#_____	Operator/Date	Verifier/Date
Obtain sterile Fetal Bovine Serum (FBS). Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____	Operator/Date	Verifier/Date
Obtain sterile Ham's F12 Medium Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____	Operator/Date	Verifier/Date
Sterilely add 74.7mL of Ham's F12 Medium to a spinner flask. Repeat with the second spinner flask 100mL spinner flask ID#_____Vol of Ham's F12_____mL 100mL spinner flask ID#_____Vol of Ham's F12_____mL	Operator/Date	Verifier/Date
Sterilely add 8.3mL ± 1 mL of FBS to each spinner flask. 100mL spinner flask ID#_____Vol of FBS_____mL 100mL spinner flask ID#_____Vol of FBS_____mL	Operator/Date	Verifier/Date
Return the inoculated spinner flask to the CO_2 incubator.	Operator/Date	Verifier/Date
Verify that CO_2 is set to 5±0.5% and that temperature is set to 37±0.5ºC. CO_2_____% Temperature _____ ºC	Operator/Date	Verifier/Date
Check media for contamination after a minimum of 24 hrs. Elapsed Incubation Time _____ 100mL spinner flask ID_____ Contamination: Y / N (Circle)	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number____________________

100mL Spinner Flask ID#___________________________________

TIME (hours)	OD 650nm	pH	LIVE CELL Count	DEAD CELL Count	Viable cells/ml	Percent Viability	GLUCOSE (mg/dl)	LACTATE (mmol/L)
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number____________________

100mL Spinner Flask ID#__________________________________

TIME (hours)	**OD 650nm**	**pH**	**LIVE CELL Count**	**DEAD CELL Count**	**Viable cells/mL**	**Percent Viability**	**GLUCOSE (mg/dl)**	**LACTATE (mmol/L)**
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

4. Solution and Buffer Preparation 500mL 1M ($NaHCO_3$) sodium bicarbonate 100mL of 1X PBS Phosphate buffered Saline		
Weigh out 21.0 ± 1 grams of ($NaHCO_3$) sodium bicarbonate. Label container: 1M $NaHCO_3$, [date], [initials], [group number], storage: room temp, disposal: drain. Balance ID #:_____ Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Dissolve $NaHCO_3$ in 250 ± 5mL of deionized water using magnetic stirrer. Volume of water added _____ mL	Operator/Date	Verifier/Date
Dilute 10 ± 0.5mL of 10X stock solution, with 90 ± 5mL of deionized water in 100mL bottle using magnetic stirrer. Label container: 1X PBS, [date], [initials], [group number], storage: room temp, disposal: drain. Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Volume of 10x PBS added:_____mL Volume of water added: _____mL	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

5. Assemble/Autoclave Bioreactor		
5.1. Assemble Vessel Stand		
Inspect the integrity of the large O-rings on the vessel stand and headplate. Replace if worn or cracked. Bioreactor ID #_____ Vessel stand O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.) Headplate O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
5.2. Assemble Headplate-Underside		
Inspect the integrity of the O-rings on the harvest tube, sparger, and the thermowell. Harvest tube O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.) Sparger O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.) Thermowell O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Attach harvest tube, sparger and thermowell. **Verify** that the sparger tube is aligned beneath the stirrer impeller.	Operator/Date	Verifier/Date
5.3. Attach Headplate to Vessel Stand.		
Place the headplate onto the vessel stand, positioning the holes on the outer edge of the headplate with the bolts on the vessel stand.	Operator/Date	Verifier/Date
Place the sample bottle assembly onto the bolt located by the 3 addition port and attach with a mill fastener.	Operator/Date	Verifier/Date
Secure the headplate with the 5 mill fasteners.	Operator/Date	Verifier/Date
5.4. Assemble Headplate – Topside		
Inspect the integrity of the O-ring in the condenser port of the headplate. Replace if worn or cracked. Condenser port O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced?: Yes / No (Circle one.)	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

Inspect the black seal at the bottom of the condenser underneath the retainer nut. Replace if worn or cracked. Condenser black seal worn or cracked? Yes / No (Circle one.) Black seal replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Attach condenser to headplate	Operator/Date	Verifier/Date
Remove protective cap from the bottom of the DO probe and inspect screen. Replace if damaged. Protective screen damaged? Yes / No (Circle one.) Protective screen replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Unscrew the membrane module from the bottom housing of the probe tip. Inspect the integrity of the O-ring. Replace if worn or cracked. O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Replenish DO electrolyte with O_2 electrolyte solution.	Operator/Date	Verifier/Date
Inspect the integrity of the O-ring at the top of the stainless steel DO probe. Replace if worn or cracked. O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Inspect the black seal at the top of the DO probe under the retainer nut. Replace if worn or cracked. Black seal worn or cracked? Yes / No (Circle one.) Black seal replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Attach DO probe to the headplate.	Operator/Date	Verifier/Date
Calibrate the pH probe. pH 7 Buffer Manufacturer:_____ Catalog number: _____ Lot number: _____ Expiration date:_____ pH 4 Buffer Manufacturer:_____ Catalog number: _____ Lot number: _____ Expiration date:_____	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

Record pH calibration values. pH 7.00 standard: pH value _____ temp_____ pH 4.00 standard: pH value _____ temp_____ Slope from the Display _____Expected value: 0.95-1.05 Offset from the Display_____Expected value: < ±0.3	Operator/Date	Verifier/Date
Inspect the integrity of the O-ring at the top of the pH probe. Replace if worn or cracked. O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Inspect the black seal at the top of the pH probe under the retainer nut. Replace if worn or cracked. Black seal worn or cracked? Yes / No (Circle one.) Black seal replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Attach pH probe to the headplate.	Operator/Date	Verifier/Date
5.5. Attach Filters and Tubing		
Place silicone tubing on the Sparger tube, Condenser top outlet, and CO2 overlay port. **Use** a small piece of silicon tubing to connect together 2 of the ports on the 3 port addition. **Connect** the pharmed tubing from the feed bottle to the 3 addition port. **Connect** the sample bottle tubing to the harvest tube.	Operator/Date	Verifier/Date
Clamp off all tubing (near the headplate) except the condenser top outlet. The condenser top outlet must remain unclamped to release pressure during autoclaving.	Operator/Date	Verifier/Date
Close all open ends with glass wool and cover with aluminum foil (including the harvest tube and sample bottle assembly tubing).	Operator/Date	Verifier/Date
Autoclave per SOP. Autoclave at 121°C for 20 minutes, using slow exhaust.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

6. Media Preparation and Addition / Run Preparation		
Add approximately 50mL of Fetal Bovine Serum and 10mL of 10mg/mL gentamycin to 950ml of Ham's F12 Medium. Pour into bioreactor. Ham's F12 Medium: Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Fetal Bovine Serum: Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount added:_____mL Gentamicin: Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount added:_____mL	Operator/Date	Verifier/Date
Verify that deionized H_2O has been added to the thermowell with the Pt-100 temperature probe. Add more if necessary.	Operator/Date	Verifier/Date
Verify that thermal blanket is wrapped around the vessel and plugged into the ADI 1025 unit.	Operator/Date	Verifier/Date
Input the following limits per the process SOP and activate the control loops. **Parameter** **Upper limit** **Set Point** **Lower limit** pH 7.3 7.2 7.1 Temperature 38 37 36 DO 52 50 48 Stirrer RPM 76 75 74	Operator/Date	Verifier/Date
Calibrate DO probe per Applikon Bioreactor Operation SOP. **Note:** Allow DO probe to polarize for at least 6 hours before performing calibration. **Record** slope: _____ Expected values are: 1.5-3.0 at 37°C or 2.0-4.0 at 25°C	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

Turn on Air supply at pump Tank pressure _____ Tank Volume_____	Operator/Date	Verifier/Date
Turn on CO_2 supply at regulator to the bioreactor Tank pressure _____ Tank Volume_____	Operator/Date	Verifier/Date
Check the media for contamination before inoculation. Contamination? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Inoculate bioreactor when the 100mL suspension culture of CHO cells reaches a concentration of about 1,000,000 cells/ml. Volume of culture added:_____	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number____________________

Applikon Bioreactor ID#____

TIME (hours)	OD 650nm	pH	LIVE CELL Count	DEAD CELL Count	Viable cells/ml	Percent Viability	GLUCOSE (mg/dl)	LACTATE (mmol/L)
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

7. Ending a Run		
Turn off each control loop. Turn off the supply of Air pump. Turn off the supply of CO_2 tank.	Operator/Date	Verifier/Date
Aseptically remove the culture through the harvest port.	Operator/Date	Verifier/Date
Clean the pH and DO probes with a 10% bleach solution and rinse with DI water. Spray with 70% IPA and pat dry with a lint-free laboratory wipe. Place protective caps on the pH probe. Place protective caps on the DO probes.	Operator/Date	Verifier/Date
Clean the bioreactor per SOP.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date
8. Harvest and Preparation of Working Cell Bank		
Using a 25mL sterile pipet, divide the 500mL suspension culture into about 20 sterile 30mL centrifuge tubes (about 25mL per tube).	Operator/Date	Verifier/Date
Centrifuge tubes for 10min at 2000rpm. (If using the Sigma 2K15 choose program 75). BE SURE TO BALANCE TUBES WHEN LOADING ROTOR.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

9. Prepare storage menstruum:		
In a container capable of holding >50mL add 40mL ± 1mL of Ham's F12 manufacturer: _____ lot number: _____ expiration date: _____ volume Ham's F12:_____	Operator/Date	Verifier/Date
Into the same container add 5mL ± 0.5mL of FBS manufacturer: _____ lot number: _____ expiration date: _____ volume FBS:_____	Operator/Date	Verifier/Date
Into the same container add 5mL ± 0.5mL of glycerol manufacturer: _____ lot number: _____ expiration date: _____ volume FBS:_____	Operator/Date	Verifier/Date
Filter sterilize and label bottle as CHO storage Menstruum with the date.	Operator/Date	Verifier/Date
Following centrifugation, decant tPA containing medium into sterile 250mL bottles. **Label** bottles as unpurified tPA in Ham's F12/FBS and date. **Store** supernatant in the refrigerator at 2-8°C.	Operator/Date	Verifier/Date
Add about 1mL of storage menstruum to each centrifuge tube to resuspend the pelleted CHO cells. **Sterilely dispense** 1mL ± 0.1mL aliquots into sterile 1.5mL cryovials. **Label** in the following manner using a cryopen: CHO (ATCC CRL-9606), [DATE], [INITIALS]. **Place** in a styrofoam tube rack, label container same as cryovials. **Store** at -85°C.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

10. Prepare Growth Curves		
Plot: **Spinner Flask** Cells/ml, glucose, and lactate vs. time (use 2 y-axes). tPA concentration and cells/ml vs. time (use 2 y-axes). Attach graphs to Batch Record. **Bioreactor** Cells/ml, % viability, and total cells vs. time (use 2 y-axes). Cells/ml, glucose, and lactate vs. time (use 2 y-axes). tPA concentration and cells/ml vs. time (use 2 y-axes). Attach graphs to Batch Record.	Operator/Date	Verifier/Date
Send samples to QC Chemistry department for ELISA and Activity Assays.	Operator/Date	Verifier/Date
Attach QC data to the batch record.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

DOWNTREAM PROCESSING: CHO CELL TPA

Diagram: tPA Downstream Process Flow Chart

Bioreactor: End of Run – conditioned medium

Centrifugation to pellet cells

Supplementation with protease inhibitors (aprotinin, leupeptin, PMSF) & Tween 80 (0.1%)

Concentration by tangential flow filtration; final volume ~20 ml + 10 ml rinse

Acidification: adjustment of pH to 5.0 (add acetic acid dropwise; transfer to Oak Ridge tubes; incubate on ice 30 min to ON; centrifugation at 10000xg, 20 min)

Further concentration via Centricon 15 (30k MW cutoff; 20+ minutes, 4000xg)

Cation Exchange Chromatography on AKTA pure system.

Analysis of fractions by ELISA.

SDS-PAGE of EOR concentrate and tPA-containing fractions.

SOP: Millipore Tangential Flow and Diafiltration Using Pellicon XL Device of tPA

Approvals:

Preparer: Jason McMillan — Date: 07JAN14
Reviewer: Dr. Margaret Bryans — Date: 08JAN14
Reviewer: Dr. David Frank — Date: 26MAR15

1. **Purpose:**
   1.1. To concentrate and perform buffer exchange of protein products using tangential flow and diafiltration processes.
2. **Scope and Applicability:**
   2.1. Applies to performing Tangential Flow Filtration with the Millipore Pellicon XL Device to concentrate and perform buffer exchange.
3. **Summary of Method:**
   3.1. Preparation of solutions
   3.2. Install resonate and permeate tubing and tank outlet valve on the Labscale 500ml Reservoir and add the stir bar
   3.3. Connect the Labscale 500 ml Stir Base to power and check operation
   3.4. Install the Pellicon XL Device
   3.5. Flush and precondition the Labscale Tangential Flow Filtration System
   3.6. Concentrate the sample, perform a buffer exchange on the sample, and then retrieve the sample.
   3.7. Flush, clean, and drain the system.
   3.8. Flush and prepare the Pellicon XL Device for storage.
   3.9. Clean the Labscale Tangential Flow Filtration System.
4. **References:**
   4.1. pH meter SOP
5. **Definitions:**
   5.1. Permeate- the material that passes through the membrane.
   5.2. Retentate- the material that does not pass through the membrane.
6. **Precautions:**
   6.1. 0.1N NaOH is very corrosive. It is extremely damaging to eyes and mucous membranes. It causes burns. Avoid contact with skin. It is harmful if swallowed or inhaled. The Millipore Pellicon XL Device is stored flat at 4-25°C with 10ml of 0.1N NaOH.
   6.2. NEVER tighten the clamp enough to completely restrict the flow in the Retentate tube. This could damage the filter and cause the tubing to disconnect.
7. **Responsibilities:**
   7.1 It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   7.2 It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
8. **Equipment and Materials:**
   8.1. 0.1N NaOH (sodium hydroxide)
   8.2. 0.05N NaOH (sodium hydroxide)

SOP: Millipore Tangential Flow and Diafiltration Using Pellicon XL Device of tPA

8.3. NaH_2PO_4 (sodium phosphate monobasic, anhydrous)
8.4. Na_2HPO_4-$7H_2O$ (sodium phosphate dibasic, heptahydrate)
8.5. preconditioning buffer
8.6. pH Meter and pH paper
8.7. 2 L filter unit
8.8. 2 magnetic stir plate and stir bars
8.9. Millipore Tangential Flow Filtration System and Pellicon XL Device and Accessories
8.10. 3 containers, 500mL
8.11. 50 mL graduated cylinder
8.12. MilliQ Water
8.13. 2L graduated cylinder
8.14. 2L Flask
8.15. 10ml graduated cylinder
8.16. 25 ml beaker

9. Procedure:

9.1. ***Preparation and Set Up***

9.1.1. Prepare 0.1N NaOH for cleaning.
9.1.1.1. Using a 1L graduated cylinder, measure 1L of MilliQ water.
9.1.1.2. Transfer water to a 1L flask.
9.1.1.3. Weigh 4.0±0.05g of NaOH.
9.1.1.4. Transfer NaOH to flask.
9.1.1.5. Add magnetic stir bar and stir to dissolve.
9.1.1.6. Sterile filter the solution and label container: 0.1N NaOH, [date], [initials], [group number], Storage: room temp, Disposal: adjust to pH 7 then drain.

9.1.2. Prepare 0.05N NaOH for Pellicon XL Device Storage
9.1.2.1 Using a 10 ml graduated cylinder, measure 5 ml of MilliQ water
9.1.2.2 Transfer MilliQ water to 25 ml beaker
9.1.2.3 Using a 10 ml graduated cylinder, measure 5 ml of 0.1N NaOH
9.1.2.4 Transfer 5 ml of 0.1N NaOH to 25 ml beaker
9.1.2.5 Add magnetic stir bar and stir to dissolve.
9.1.2.6 Sterile filter the solution and label container: 0.05N NaOH, [date], [initials], [group number], Storage: room temp

9.1.3 Diafiltration Buffer Preparation (20mM Phosphate Buffer pH 7.1)
9.1.3.1 Weigh out 0.80±0.02g NaH_2PO_4 and place into 1L flask
9.1.3.2 Weigh out 3.60±0.2g Na_2HPO_4-$7H_2O$ and place into the 1L flask containing NaH_2PO_4.
9.1.3.3 Using a 1L graduated cylinder, measure 1L of MilliQ water.
9.1.3.4 Add the water to the 1L flask containing the phosphates.
9.1.3.5 Add a magnetic stir bar and stir to dissolve.
9.1.3.6 Adjust pH to 7.1±0.1.
9.1.3.7 Sterile filter the solution and label container: 20mM Phosphate Buffer pH 7.1, [date], [initials], [group number], Storage: room temp, Disposal: drain

SOP: Millipore Tangential Flow and Diafiltration Using Pellicon XL Device of tPA

9.2. ***Labscale 500ML Reservoir Set Up***

9.2.1 **Install Retenate tubing**

Note: All tubing lengths are recommended to minimize recirculation volume. Longer lengths may be used. After prolonged storage, the tubing may absorb a small volume of water. As a result, the tubing color may change from translucent to opaque, which is normal. Air or oven drying will return the color to translucent.

9.2.1.1. Cut silicone (translucent) tubing and install fittings as shown in figure 10.

9.2.1.2. Remove plugs from retenate outlet (RET OUT) and retenate inlet (RET IN) ports.

9.2.1.3. Insert the male luer end of the retenate tubing into the RET OUT port and the female luer end of the retenate tubing into the RET IN port. Turn fittings until snug.

9.2.2. **Install Permeate tubing**

9.2.2.1. Cut silicone (translucent) tubing and install fittings as shown in figure 12.

9.2.2.2. Remove the plug from the permeate outlet port (PERM 2) and insert the male luer end of the permeate silicone (translucent) tubing into the PERM2 port. Turn fittings until snug.

9.3. ***Install Tank Outlet Valve***

9.3.1. Remove plug from the tank outlet port (TANK OUT) and insert the female luer end of the tank outlet valve over the TANK OUT port. Turn the lock nut until snug.

9.3.2. Install Vent Filter (If required)

9.3.3. If a sterile vent is required, remove plug from the vent (VENT) port and insert the male luer end of MILLEX filter into the vent port.

9.4. ***Install Stir Bar***

9.4.1. If mixing is required, open reservoir cover and drop stir bar to the bottom of the reservoir.

9.5. ***Labscale Stir Base Set Up***

9.5.1. **Power Connection**

9.5.1.1. Turn Stirrer and pump speed controls to the off position.

9.5.1.2. Connect power cord to the power cord receptacle located at the rear of the system base.

9.5.1.3. Align detent on connector with receptacle.

9.5.1.4. Press connector into receptacle and turn lock ring to secure.

9.5.2. **Check Operation**

9.5.2.1. Remove the plugs from the pump inlet and pump outlet ports.

9.5.2.2. Turn on the pump speed control, set to 2, and listen for pump motor.

9.5.2.3. Turn off the pump speed control.

9.5.2.4. Turn on the stirrer speed control and listen for the stirrer motor.

9.5.2.5. Turn off the stirrer speed control.

9.6. ***Install Pellicon XL Device***

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9.6.1. Remove the plugs from FEED, RET, PERM 1, and PERM 2 ports on the Pellicon XL Device.

9.6.2. Align the Pellicon XL device ports with Labscale 500 ml Reservoir ports being sure the PERM and RET DEVICE ports of the Pellicon XL Device and reservoir match. Press the device firmly onto the reservoir ports. Turn the lock nuts until snug.

9.7. ***Flushing***

9.7.1. Disconnect retenate silicone (translucent) tubing from RET IN port and place end of retenate tubing in waste collection vessel.

9.7.2. Place end of permeate silicone (translucent) tubing into waste collection vessel. Open retenate valve by turning the counterclockwise.

9.7.3. Remove the reservoir cover and fill reservoir with 500 ml of MilliQ water. Remove the plug from VENT port and open tank outlet valve.

9.7.4. Turn the pump on and increase the speed until the feed pressure gauge reads 1.38 Bar (20 psi).

9.7.5. Continue pumping to the waste collection vessel until the level in the reservoir drops to 350 ml and then turn the pump off.

9.7.6. Reconnect the retenate silicone (translucent) tubing to the RET IN port and turn the pump on. Slowly increase the pump speed until feed pressure gauge reads 1.38 Bar (20 psi). Check the system for leaks and tighten connections if leaks are found.

9.7.7. Adjust retenate valve restriction by slowly turning retenate valve clockwise until the retenate pressure gauge reads 0.69 Bar (10 psi).

9.7.8. Adjust pump speed and retenate valve restriction to achieve 2.07 Bar (30 psi) feed pressure and 0.69 Bare (10 psi) retenate pressure.

9.8. ***Pre-conditioning***

9.8.1. Place end of permeate tubing silicone (translucent) in the waste collection vessel.

9.8.2. Remove reservoir cover and fill the reservoir with 50 ml of an appropriate buffer and then remove the Vent port plug.

9.8.3. Open the tank outlet valve. Turn the pump on and increase the pump speed until the feed pressure gauge reads 1.38 Bar (20 psi). Check all system connections for leaks and tighten any connections as necessary.

9.8.4. Continue pumping to the waste connection vessel until the level in the reservoir drops to the bottom of the reservoir stir bar well making sure to stop the pump before air is pumped into the system. Turn the pump off.

9.9. ***Drain Permeate Tubing***

9.9.1. Disconnect the permeate tubing (silicone, translucent) from the permeate outlet (Perm 2) port. Drain the permeate tubing into the waste collection vessel.

9.9.2. Reconnect the permeate tubing to the PERM 2 port.

9.10. ***Concentrate the Sample***

9.10.1. Remove the reservoir cover and fill the reservoir with tPA sample to be concentrated.

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9.10.2. Ensure the vent port is open by removing the plug from the VENT port and leaving it open or installing a Millex Filter if required. Open the tank outlet valve.
9.10.3. Turn the pump on and increase the pump speed until the feed pressure gauge reads 1.38 Bar (20 psi). Check all system connections for leaks and tighten any connections as necessary.
9.10.4. Adjust the retenate valve restriction by slowly turning the retenate valve clockwise until the retenate pressure gauge reads 0.69 Bar (10 psi).
9.10.5. Adjust the pump speed and retenate valve restriction to achieve desired feed retenate pressures (2.07 Bar (30 psi feed / 0.69 Bar (10 psi) retenate). Do not exceed 4.14 Bar (60 psi) feed pressure.
9.10.6. Filter the solution until the desired volume is reduced 10 fold.
9.10.7. Turn off the pump and empty the permeate container into a large bottle with a cap and label ad: tPA Permeate Waste, disposal; bleach then drain, [initials], [date].

9.11. ***Perform a Buffer Exchange on the Sample***

9.11.1. Add the 20mM Phosphate Buffer to the sample to bring the volume back to the pre-concentrated volume.
9.11.2. Repeat Concentrate the Sample (Number will go here) until the pH of the concentrated retenate is 7.1 ±0.1 as measured with a pH meter.

9.12. ***Retrieve the Sample***

9.12.1. Disconnect the pump outlet tubing (Sta-Pure, white) from pump outlet port and place in product recovery collection vessel.
9.12.2. Disconnect the retenate tubing (silicone, translucent) from the retenate in port. Fluid should now drain by gravity. If additional drainage is required, a syringe can be placed on the end of the retenate tube and fluid can be blown down.
9.12.3. Replace retenate tubing (silicone, translucent) in retenate port. Reconnect pump outlet tubing (Sta-Pure, white).
9.12.4. Disconnect FEED IN tubing and place in collection vessel. Open tank outlet valve, turn pump speed up to drain reservoir.
9.12.5. Reconnect the pump outlet tubing (Sta-Pure, white) to the Feed In port.
9.12.6. Label the recovery collection vessel Concentrated tPA, [date], [initials].
9.12.7. Store in 2°C-8°C refrigerator for use in further purification steps.

9.13. ***Flushing***

9.13.1. Repeat Flushing as described in 9.7

9.14. ***Cleaning***

9.14.1. Disconnect the retenate tubing (silicone, translucent) from RET IN port and place in waste collection vessel. Place the end of the permeate tubing in the waste collection vessel.
9.14.2. Open the retenate valve by turning it counterclockwise.
9.14.3. Remove the reservoir cover and fill with 500 ml of 0.1N NaOH. Ensure the vent port is open by removing the plug from the VENT port and either leave open or install a Millex Filter.
9.14.4. Open the tank outlet valve.

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9.14.5. Turn the pump on and increase the pump speed until the feed pressure gauge reads 1.38 Bar (20 psi). Check all system connections for leaks and tighten any connections as necessary.

9.14.6. Continue pumping to the waste collection vessel until the level in the reservoir drops to 250 ml and then turn the pump off. Reconnect the retenate (silicone, translucent) tubing to the RET IN port.

9.14.7. Connect the male luer end of the permeate tubing to the recirculation (DIA / RECIRC) port. Turn the pump on and increase the pump speed until the feed pressure gauge reads 1.38 Bar (20 psi). Check all system connections for leaks and tighten any connections as necessary.

9.14.8. Adjust the retenate valve restriction by slowly turning the retenate valve clockwise until the retenate pressure gauge reads 0.69 Bar (10 psi). Adjust the pump speed and retenate valve restriction to achieve 2.07 Bar (30 psi) feed pressure and 0.69 Bar (10 psi) retenate pressure.

9.14.9. Recirculate the cleaning solution for 30-60 minutes and then turn the pump off.

9.15. ***Drain the System***

9.15.1. Disconnect the pump outlet (Sta-pure, white) tubing from the pump outlet port and place in waste collection vessel.

9.15.2. Disconnect the retenate silicone (translucent) tubing from the RET IN port. Fluid should now drain by gravity. If additional drainage is required, a syringe can be placed on the end of the retenate tube and fluid can be blown down.

9.16. ***Flushing***

9.16.1. Repeat Flushing as described in 9.7

9.17. ***Pellicon XL Device Storage***

9.17.1. Turn all of the lock nuts until you are able to remove the Pellicon XL Device.

9.17.2. Fill a syringe with 0.05N NaOH Storage solution.

9.17.3. Attach the syringe to the retenate port and slowly push the solution into the device. Remove the syringe and replace all of the plugs on the ports and store flat at 4°C-25°C.

9.18. ***Clean Base***

9.18.1. Disconnect the power cord.

9.18.2. Clean exterior surfaces, reservoir, and Labscale System Base with a mild soap and water solution.

10. Attachments:

SOP: Millipore Tangential Flow and Diafiltration Using Pellicon XL Device of tPA

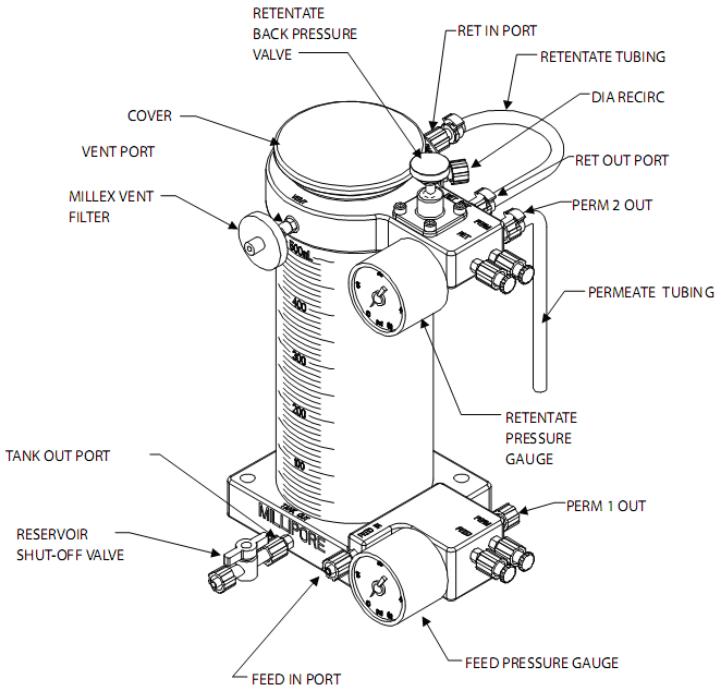
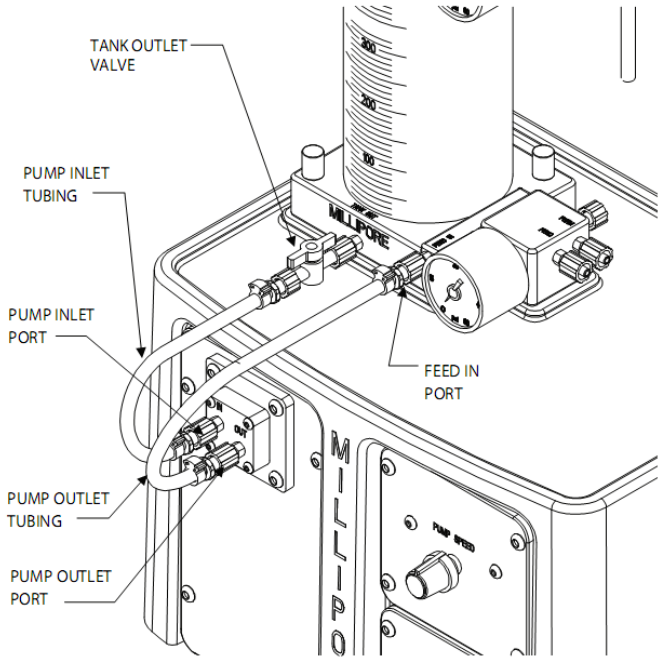


Figure 1: Reservoir Set Up
(http://www.millipore.com/userguides.nsf/docs/p60085)



SOP: Millipore Tangential Flow and Diafiltration Using Pellicon XL Device of tPA

Figure 2: Pump Base Set Up
(http://www.millipore.com/userguides.nsf/docs/p60085)

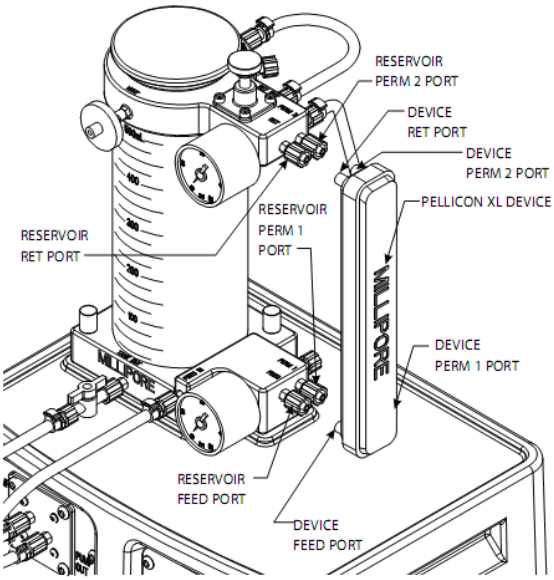


Figure 3: Installation of Pellicon XL Device
(http://www.millipore.com/userguides.nsf/docs/p60085)

11. History

Revision Number	*Effective Date*	*Preparer*	*Description of Change*
0	07JAN14	Jason McMillan	Initial release

SOP: End-of-Run tPA Process: Harvest, Centrifugation, Concentration, pH Adjustment

Approvals:

Preparer: Jason McMillan & Dr. David Frank — Date: 15APR15
Reviewer: Dr. Maggie Bryans — Date: 15APR15

1. **Purpose:**
   1.1. To harvest t-PA-containing conditioned medium and prepare for purification of t-PA by TFF concentration and pH adjustment; intermediate steps include centrifugation to remove cells prior to tangential flow filtration and to pellet precipitated protein following pH adjustment.
2. **Scope and Applicability:**
   2.1. A biomanufacturing environment requires proper steps to recover and purify active pharmaceutical ingredient from a bioreactor or fermentor. This SOP provides bench scale procedures to accomplish that goal using conditioned medium from cells expressing recombinant tissue-type plasminogen activator. The method demonstrates the principles of tangential flow filtration, centrifugation, and pH dependent protein precipitation in preparation for downstream processing by column chromatography as may be employed in a typical process development, for later scale up to manufacturing.
3. **Summary of Method:**
   3.1. Preparation of solutions:
      3.1.1. PBS/Tween 80 for preconditioning of the Pellicon cassette (for TFF)
      3.1.2. 0.1 N NaOH for cleaning the Pellicon cassette following use
      3.1.3. 0.05 N NaOH for storage of the Pellicon cassette
   3.2. Flushing and preconditioning of TFF/Pellicon.
   3.3. Transfer of culture from bioreactor to centrifuge bottles.
   3.4. Centrifugation to pellet cells.
   3.5. Transfer of conditioned medium (CM) from centrifuge bottle to storage vessel/bottle.
   3.6. Addition of protease inhibitors and Tween 80.
   3.7. Concentration of supplemented CM by tangential flow filtration.
   3.8. Adjustment of pH of concentrated CM.
   3.9. Centrifugation.
4. **References:**
   4.1 Millipore Tangential Flow and Diafiltration Using Pellicon XL Device of tPA SOP
   4.2 Oakton PC 700 Bench Series pH/Conductivity/°C/°F Meter SOP (Doc # 1.0).
5. **Definitions:**
   5.1. Permeate- the material that passes through the membrane.
   5.2. Retentate- the material that does not pass through the membrane.
   5.3. TFF – tangential flow filtration
   5.4. CM – conditioned medium, which contains the API of interest

SOP: End-of-Run tPA Process: Harvest, Centrifugation, Concentration, pH Adjustment

6. Precautions:

6.1. 0.1N NaOH is very corrosive. It is extremely damaging to eyes and mucous membranes. It causes burns. Avoid contact with skin. It is harmful if swallowed or inhaled. The Millipore Pellicon XL Device is stored flat at 4-25°C with 10ml of 0.1N NaOH.

6.2. Acetic acid vapor is extremely irritating to the airways upon inhalation, and should therefore be used only in the fume hood.

6.3. NEVER tighten the clamp enough to completely restrict the flow in the Retentate tube. This could damage the filter and cause the tubing to disconnect.

6.4. Luer Lock fittings on the TFF device should be tightened with care not to exert too much force, to avoid stripping threads or damaging the fitting.

7. Responsibilities:

7.1 It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.

7.2 It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

8. Equipment and Materials:

8.1. 250 ml Nalgene centrifuge bottles (3)
8.2. 250 ml Corning bottles (3)
8.3. 0.1N NaOH (sodium hydroxide)
8.4. 0.05N NaOH (sodium hydroxide)
8.5. acetic acid, glacial
8.6. 10% (w/v) Tween 80
8.7. NaH_2PO_4 (sodium phosphate monobasic, anhydrous), or pre-made PBS (phosphate buffered saline)
8.8. Na_2HPO_4-$7H_2O$ (sodium phosphate dibasic, heptahydrate), or pre-made PBS
8.9. preconditioning buffer (PBS containing 0.1% Tween 80)- 50 ml
8.10. Stock solutions of protease inhibitors:
  8.10.1. 10 mg/ml PMSF (phenylmethylsulfonylflouride); 250X
  8.10.2. Leupeptin, 2 mg/ml; 4000x
  8.10.3. Aprotinin, 10 mg/ml, 5000x
8.11. pH Meter and pH paper
8.12. 2 magnetic stir plate and stir bars
8.13. Millipore Tangential Flow Filtration System and Pellicon XL Device and Accessories
8.14. MilliQ Water
8.15. 10ml graduated cylinder
8.16. 25 ml beaker

9. Procedure:

9.1. ***Preparation and Set Up***

9.2. Preparation of solutions (provided).
  9.2.1. Prepare 0.1N NaOH for cleaning.
    9.2.1.1. Using a 1L graduated cylinder, measure 1L of MilliQ water.
    9.2.1.2. Transfer water to a 1L flask.

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9.2.1.3. Weigh 4.0±0.05g of NaOH.
9.2.1.4. Transfer NaOH to flask.
9.2.1.5. Add magnetic stir bar and stir to dissolve.
9.2.1.6. Sterile filter the solution and label container: 0.1N NaOH, [date], [initials], [group number], Storage: room temp, Disposal: adjust to pH 7 then drain.

9.2.2. Prepare 0.05N NaOH for Pellicon XL Device Storage
9.1.2.1 Using a 10 ml graduated cylinder, measure 5 ml of MilliQ water
9.1.2.2 Transfer MilliQ water to 25 ml beaker
9.1.2.3 Using a 10 ml graduated cylinder, measure 5 ml of 0.1N NaOH
9.1.2.4 Transfer 5 ml of 0.1N NaOH to 25 ml beaker
9.1.2.5 Add magnetic stir bar and stir to dissolve.
9.1.2.6 Sterile filter the solution and label container: 0.05N NaOH, [date], [initials], [group number], Storage: room temp

9.3. ***Labscale 500ML Reservoir Set Up***

9.4. Flushing and preconditioning of TFF/Pellicon. One should become familiar with the location of ports and tubing connection points as shown in the attachments at the end of this SOP prior to beginning setup.

9.4.1. Set up the apparatus and confirm that all tubing connections are secure, according to the SOP (Millipore Tangential Flow and Diafiltration Using Pellicon XL Device of tPA SOP).

9.4.2. Remove the 4 plugs on the Pellicon cassette and attach the Pellicon cassette to the Labscale apparatus.

9.4.3. Add 500 ml MilliQ water to the reservoir and flush the cassette as described in section 9.4.4.

9.4.4. ***Flushing the Pellicon cassette.***

9.4.4.1. Disconnect retentate silicone (translucent) tubing from RET IN port and place end of retentate tubing in waste collection vessel.
9.4.4.2. Place end of permeate silicone (translucent) tubing into waste collection vessel. Open retentate valve by turning the counterclockwise.
9.4.4.3. Remove the reservoir cover and fill reservoir with 500 ml of MilliQ water. Remove the plug from VENT port and open tank outlet valve.
9.4.4.4. Turn the pump on and increase the speed until the feed pressure gauge reads 1.38 Bar (20 psi).
9.4.4.5. Continue pumping to the waste collection vessel until the level in the reservoir drops to 350 ml and then turn the pump off.
9.4.4.6. Reconnect the retentate silicone (translucent) tubing to the RET IN port and turn the pump on. Slowly increase the pump speed until feed pressure gauge reads 1.38 Bar (20 psi). Check the system for leaks and tighten connections if leaks are found.
9.4.4.7. Adjust retentate valve restriction by slowly turning retentate valve clockwise until the retentate pressure gauge reads 0.69 Bar (10 psi).

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9.4.4.8. Adjust pump speed and retentate valve restriction to achieve 2.07 Bar (30 psi) feed pressure and 0.69 Bare (10 psi) retentate pressure.

9.4.4.9. Allow to run until 50 ml remains in the chamber.

9.4.4.10. Disconnect the pump outlet (Sta-pure, white) tubing from the pump outlet port and place in waste collection vessel.

9.4.4.11. Disconnect the retentate silicone (translucent) tubing from the RET IN port. Fluid should now drain by gravity. If additional drainage is required, a syringe can be placed on the end of the retentate tube and fluid can be blown down.

9.4.4.12. Remove the remainder of water in the chamber as follows: Replace retentate tubing (silicone, translucent) in retentate port. Reconnect pump outlet tubing (Sta-Pure, white).

9.4.4.13. Disconnect FEED IN tubing and place in collection vessel. Open tank outlet valve, turn pump speed up to drain reservoir.

9.4.4.14. Reconnect the pump outlet tubing (Sta-Pure, white) to the Feed In port.

9.5. ***Pre-conditioning***

9.5.1. Place end of permeate tubing silicone (translucent) in the waste collection vessel.

9.5.2. Remove reservoir cover and fill the reservoir with 50 ml of PBS containing 0.1% Tween 80 (or other appropriate buffer) and then remove the Vent port plug.

9.5.3. Open the tank outlet valve. Turn the pump on and increase the pump speed until the feed pressure gauge reads 1.38 Bar (20 psi at its maximum; the needle will pulse as the pump turns). Check all system connections for leaks and tighten any connections as necessary.

9.5.4. Continue pumping to the waste collection vessel until the level in the reservoir drops to the bottom of the reservoir stir bar well making sure to stop the pump before air is pumped into the system. Turn the pump off.

9.6. ***Transfer of culture from bioreactor to centrifuge bottles.***

9.6.1. Refer to the SOP: Applikon ez-Control Bioreactor Controller Operation for instructions on removing the headplate of the bioreactor, providing access to the cells and conditioned medium.

9.6.2. Transfer the culture to three 250 ml centrifuge bottles using a 50 ml pipet and PipetAid. Residual culture can be transferred to an Ehrlenmeyer flask for temporary storage.

9.6.3. Centrifuge cells in pre-chilled Sorvall centrifuge, fitted with a SLA1500 rotor, at 500 x *g* for 5 min, 4 degrees C.

9.6.4. Transfer conditioned medium (CM) from centrifuge bottle to storage vessel/bottle by carefully decanting the supernatant to appropriately labeled 250 ml Corning bottles.

9.6.5. Add protease inhibitors and Tween 80 as follows. To each 250 ml bottle of CM supernatant, add 1 ml 10mg/ml PMSF, 50 µl of 10 mg/ml Aprotinin stock and 62.5 µl 2 mg/ml Leupeptin stock. Also add 2.5 ml 10% Tween 80 (final concentration will be 0.1%).

SOP: End-of-Run tPA Process: Harvest, Centrifugation, Concentration, pH Adjustment

9.7. ***Concentrate the Sample***

9.7.1. Remove the reservoir cover and fill the reservoir with tPA sample (up to 500 ml) to be concentrated.

9.7.2. Ensure the vent port is open by removing the plug from the VENT port and leaving it open or installing a Millex Filter if required. Open the tank outlet valve.

9.7.3. Turn the pump on and increase the pump speed until the feed pressure gauge reads 1.38 Bar (20 psi). Check all system connections for leaks and tighten any connections as necessary.

9.7.4. Adjust the retentate valve restriction by slowly turning the retentate valve clockwise until the retentate pressure gauge reads 0.69 Bar (10 psi).

9.7.5. Adjust the pump speed and retentate valve restriction to achieve desired feed retentate pressures [2.07 Bar (30 psi feed / 0.69 Bar (10 psi) retentate]. Do not exceed 4.14 Bar (60 psi) feed pressure.

9.7.6. Filter the solution until the desired volume is reduced 10 fold or greater, but ideally down to about 20 ml.

9.7.7. Turn off the pump and empty the permeate container into a large bottle with a cap and label as: tPA Permeate Waste, disposal; bleach then drain, [initials], [date].

9.8. ***Retrieve the Sample***

9.8.1. Disconnect the pump outlet tubing (Sta-Pure, white) from pump outlet port and place in product recovery collection vessel (beaker with small stir bar is preferred; or 50 ml tube).

9.8.2. Disconnect the retentate tubing (silicone, translucent) from the retentate in port and open back pressure regulation valve (turn counterclockwise). Fluid should now drain by gravity.

9.8.3. When drainage ceases, rinse the Pellicon innards by injection of 5 ml PBS/0.1% Tween 80 from the retentate tube using a 10 ml syringe. Additional drainage is required; a syringe can be placed on the end of the retentate tube and fluid can be blown down.

9.8.4. Replace retentate tubing (silicone, translucent) in retentate port. Reconnect pump outlet tubing (Sta-Pure, white).

9.8.5. Disconnect FEED IN tubing and place in collection vessel. Open tank outlet valve, turn pump speed up to drain reservoir.

9.8.6. Stop the pump, close the outlet valve, and add 5 ml PBS/Tween80 to the chamber to rinse sides and effect collection of residual tPA. Pipet the solution along the walls repeatedly to rinse, then collect and transfer to the collection vessel.

9.8.7. Reconnect the pump outlet tubing (Sta-Pure, white) to the Feed In port.

9.8.8. Label the recovery collection vessel Concentrated tPA, [date], [initials].

9.9. ***Adjustment of pH of concentrated Conditioned Medium (CM)***.

Glacial acetic acid is irritating to the airways upon inhalation. Therefore perform the following operation in the fume hood with the sash as low as is comfortably possible to work.

SOP: End-of-Run tPA Process: Harvest, Centrifugation, Concentration, pH Adjustment

9.9.1. Place the beaker containing concentrate on a stir plate, insert pH probe and monitor pH.
9.9.2. Add glacial acetic acid dropwise to the concentrated tPA to reduce the pH to 5.0 +/- 0.1.
9.9.3. Transfer the mixture to the minimum number of Oak Ridge centrifuge tubes necessary to contain it (capacity of each tube is about 40 ml).
9.9.4. Leave the mixture on ice for at least 30 min (overnight at 4°C is acceptable, and offers a convenient stopping point) to allow formation of precipitate.

9.10. ***Clarification of pH 5.0 Solution by Centrifugation***

9.10.1. Centrifuge pH 5.0 mixture at 10,000 x *g* for 10 minutes, 4°C.
9.10.2. Transfer the supernatant to appropriately sized tube(s) labeled tPA-TFF/pH 5.
9.10.3. Store in 2°C-8°C refrigerator for use in further purification steps.

9.11. ***Flushing***

9.11.1. To begin cleaning the Millipore TFF apparatus and Pellicon filter, repeat Flushing as described in 9.4.4

9.12. ***Cleaning the Labscale TFF/Pellicon cassette.***

9.12.1. Disconnect the retentate tubing (silicone, translucent) from RET IN port and place in waste collection vessel. Place the end of the permeate tubing in the waste collection vessel.
9.12.2. Open the retentate valve by turning it counterclockwise.
9.12.3. Remove the reservoir cover and fill with 500 ml of 0.1N NaOH. Ensure the vent port is open by removing the plug from the VENT port and either leave open or install a Millex Filter.
9.12.4. Open the tank outlet valve.
9.12.5. Turn the pump on and increase the pump speed until the feed pressure gauge reads 1.38 Bar (20 psi). Check all system connections for leaks and tighten any connections as necessary.
9.12.6. Continue pumping to the waste collection vessel until the level in the reservoir drops to 250 ml and then turn the pump off. Reconnect the retentate (silicone, translucent) tubing to the RET IN port.
9.12.7. Connect the male luer end of the permeate tubing to the recirculation (DIA / RECIRC) port. Turn the pump on and increase the pump speed until the feed pressure gauge reads 1.38 Bar (20 psi). Check all system connections for leaks and tighten any connections as necessary.
9.12.8. Adjust the retentate valve restriction by slowly turning the retentate valve clockwise until the retentate pressure gauge reads 0.69 Bar (10 psi). Adjust the pump speed and retentate valve restriction to achieve 2.07 Bar (30 psi) feed pressure and 0.69 Bar (10 psi) retentate pressure.
9.12.9. Recirculate the cleaning solution for 30-60 minutes and then turn the pump off.

9.13. ***Drain the System***

9.13.1. Disconnect the pump outlet (Sta-pure, white) tubing from the pump outlet port and place in waste collection vessel.

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9.13.2. Disconnect the retentate silicone (translucent) tubing from the RET IN port. Fluid should now drain by gravity. If additional drainage is required, a syringe can be placed on the end of the retentate tube and fluid can be blown down.

9.14. ***Flushing***

9.14.1. Repeat Flushing as described in 9.4.4.

9.15. ***Pellicon XL Device Storage***

9.15.1. Turn all of the lock nuts until you are able to remove the Pellicon XL Device.

9.15.2. Fill a 10 ml syringe with 0.05N NaOH Storage solution.

9.15.3. Place the cassette in sink or tray that can contain any overflow. Attach the syringe to the retentate port and slowly push the solution into the device. Remove the syringe and replace all of the plugs on the ports and store flat at 4°C-25°C.

9.16. ***Clean Base***

9.16.1. Disconnect the power cord.

9.16.2. Clean exterior surfaces, reservoir, and Labscale System Base with a mild soap and water solution.

10. Attachments:

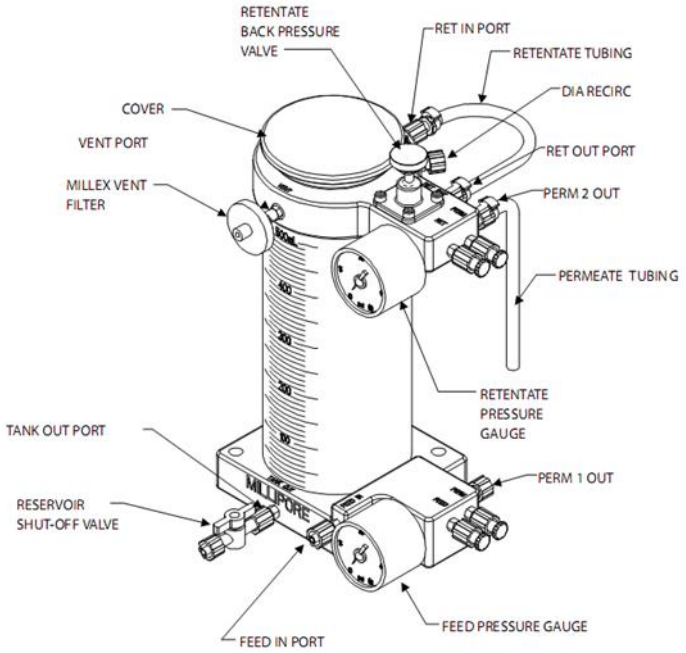

Figure 1: Reservoir Set Up
(http://www.millipore.com/userguides.nsf/docs/p60085)

SOP: End-of-Run tPA Process: Harvest, Centrifugation, Concentration, pH Adjustment

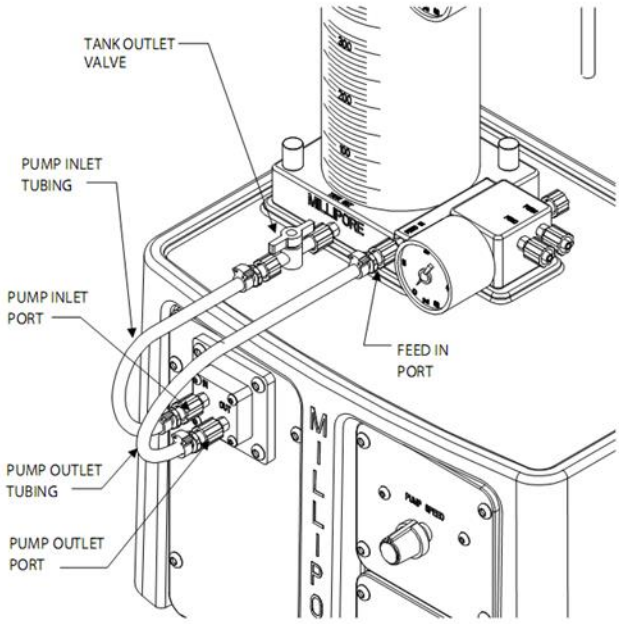

Figure 2: Pump Base Set Up
(http://www.millipore.com/userguides.nsf/docs/p60085)

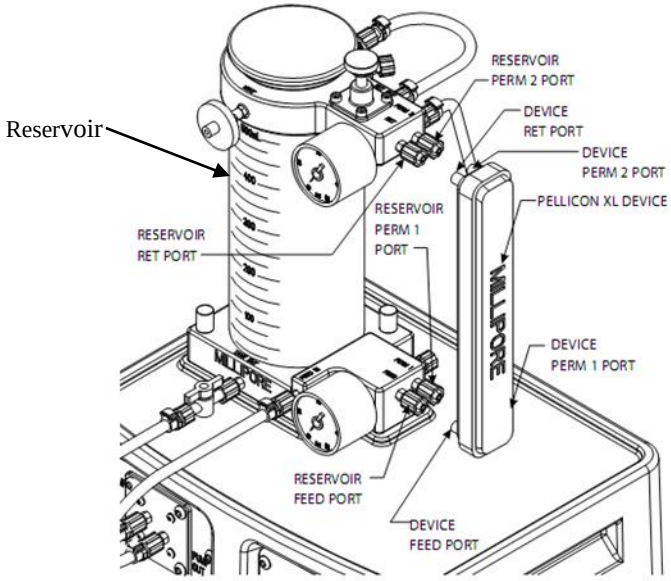

Figure 3: Installation of Pellicon XL Device
(http://www.millipore.com/userguides.nsf/docs/p60085)

SOP: End-of-Run tPA Process: Harvest, Centrifugation, Concentration, pH Adjustment

11. History

Revision Number	***Effective Date***	***Preparer***	***Description of Change***
0	07JAN14	Jason McMillan	Initial release

Batch Process Record: tPA Production from CHO Cells: Chromatography Operation

1.0 Description

1.1 This batch production record covers the precise operating steps necessary to purify recombinant tissue-type plasminogen activator from concentrated conditioned cell culture medium using cation exchange chromatography.

2.0 Reference

Title	Document Number
TFF ref. SOP	DP 2
Supplementation/Acidification/Precipitation	
GE Healthcare AKTA pure Equipment SOP	DP 5
SOP for Bradford Protein Assay	
SOP for tPA ELISA	QCB 1

3.0 Equipment

Equipment Type	Manufacturer, Model Number	Calibration Due Date	Initials/Date	Verifier/Date
Chromatography System	GE Healthcare AKTApure			
Column	HiTrap SP, 5ml	N/A		

4.0 Components

Component	Quantity Required	Quantity Used	Initials/Date	Verifier/Date
Fraction tubes	50			
Syringe, 1 ml	1			
Ehrlenmeyer flask, 125 ml	1			
Ehrlenmeyer flask, 1L	1			

5.0 Solutions

Solution	ID	Date Prepared	Volume Required	Volume Used	Initials/ Date
Start Buffer	0.2M NaOAc, pH 5, 0.1% Tween 80		250 ml		
End Buffer	0.2M NaOAc, pH 5, 0.1% Tween 80, 1M NaCl		250 ml		
Filtered, degassed MilliQ			500 ml		

Batch Process Record: tPA Production from CHO Cells: Chromatography Operation

water					

6.0 Procedure

6.1 Chromatography system setup

#	Task	Performed Initial/Date	Verified Initial/Date
1	Place or verify that Buffer A is in place, securely located atop the instrument. Insert tubing for inlet A1 to the bottom of the container. Approximate volume of Buffer A:_____ ml		
2	Place or verify that the Buffer B container is in place, securely located atop the instrument. Insert tubing for inlet B1 to the bottom of the container. Approximate volume of Buffer B:_____ ml		
3	Verify that the tubing labelled Outlet is placed into a 125 ml E. flask		
4	Verify that the Waste effluent tubing labeled W, W1, and W2, are placed in a 1L E flask		
5	Confirm that an adequate supply (50) of tubes are placed in the fraction collector carousel.		
6	Rotate the tube carousel so that the #1 position is set to receive the initial drops. Lift the arm and swing it over to rest against the side of the first tube.		
7	Load a 5 ml syringe with Buffer A, removing air bubbles, and flush the 0.5 ml sample loop by injecting the entire volume		
8	Turn the AKTApure system on. The on/off switch is on the right side toward the rear of the instrument.		
9	Turn on the computer and login		
10	Open the Unicorn 6.3 software by:		

Batch Process Record: tPA Production from CHO Cells: Chromatography Operation

	1) double clicking the desktop icon 2) clicking ‘OK’ at the Log On-Unicorn dialog box		
11	Confirm that the installed column is a HiTrap SP HP 5 ml		

Batch Process Record: tPA Production from CHO Cells: Chromatography Operation

6.2 Column conditioning

#	Task	Performed Initial/Date	Verified Initial/Date
11	Equilibrate system and column. 1)Navigate to the System Control window. 2) If the window is blank, choose menu item System\Connect to System and choose OK 2) In the File menu, select Open\HiTrap SP 5ml Equilibration 3) Click Next until the Start button is shown, then choose it. 4) Allow the method to run to completion (about 15 minutes).		
12	Verify that eluent is directed into the waste flask		
13	Empty waste flask when the method is complete, then return it.		

6.3 tPA Chromatography

#	Task	Performed Initial/Date	Verified Initial/Date
1	Record the sample information. Sample origin: Batch #: Date prepared: Volume: pH:		
2	Centrifuge the sample at 10000 x g for 5 min.		
3	Sample injection into 0.5 ml sample loop: 1) Fill 1 ml syringe with sample, being careful to avoid precipitated matter and eliminate any air bubbles 2) Dispense excess sample back into its original container, retaining 0.6 ml in the syringe 3) Insert syringe firmly into sample inlet port (refer to diagram 1).		

Batch Process Record: tPA Production from CHO Cells: Chromatography Operation

	4) Inject into the 0.5 ml sample loop		
4	Initiate the run: 1)Using the Unicorn 6.3 software, open the System Control window. 2)Under the File menu, choose Open*HiTrapSP tPA Production* 3) In the resulting dialog box, input Sample Info into the designated cell. 4) Enter 5) Click Next until the Start button is shown in the dialog box. 6) Click Start to begin the separation process.		

Chromatographic run sequence is as follows:

1) Inject entire contents of 0.5 ml sample loop; begin collecting 5 ml fractions.

2) Wash unbound proteins through with up to 12 column volumes (CV) buffer A, until A280 stabilizes; eluent directed to waste.

3) Elute bound protein (mostly contaminants, with minor amount of t-PA) with linear gradient of 0-0.2M NaCl in 5CV; collect 5 ml fractions.

4) Hold at 0.2M NaCl for 2CV.

5) Elute bound t-PA with 0.5M NaCl in 2 CV; collect 2 ml fractions.

6) Flush column with 2 CV of 1.0M NaCl; collect 5 ml fractions.

7) Re-equilibrate column in 5 CV buffer A.

6.4 Evaluate Chromatographic Separation

#	Task	Performed Initial/Date	Verified Initial/Date
1	Open chromatogram (should be the most recent one listed) in Unicorn "Evaluation" tool as follows: 1) In Unicorn 6.3 software, under the Tools menu, choose Evaluation. 2) In the Evaluation window, click the Results tab. 3) Find yours in the listed chromatograms, then double click to display it in the right frame.		
2	*Optional:* Customize chromatogram: 1)Open Customize tool 2)Accept the default, or select curves for UV, conductivity, fractions;		

Batch Process Record: tPA Production from CHO Cells: Chromatography Operation

	3)adjust Y axis values for optimum display of curves		
3	*Optional.* Determine protein content per fraction by Bradford Protein Estimation. Refer to the SOP for that procedure.		
4	Use Operations\Fraction Histogram to indicate average protein content per fraction.		
5	Use Operations\Activity Histogram to enter □g tPA amount per fraction, as determined using the tPA ELISA; assay 1 □l from even numbered fractions XX-YY; refer to the tPA ELISA SOP.		
6	Save and Print: Save the chromatogram as a pdf: 1) While displaying finished chromatogram, choose File\Print 2) In the resulting dialog box, choose Preview 3) In the window that opens, click File\Save as PDF 4) Enter a name which refers to the sample, column and date (e.g. tPA from TFF on HiTrap SP 09APR15) 5) Print a copy of the chromatogram for record keeping		
7	Save changes.		

Equipment SOP: Operation of ÄKTA pure Chromatography System

Approvals

Preparer: Dr. David Frank — Date: 16JUL15
Reviewer: Jason McMillan — Date: 16JUL15
Reviewer: Dr. Maggie Bryans — Date: 16JUL15

1. **Purpose**
   1.1. This procedure describes the operation of the ÄKTA pure Chromatography System, controlled by Unicorn 6.3 software.
2. **Scope and Applicability**
   2.1. Applies to chromatography of proteins, etc using a column installed on the GE ÄKTA pure Chromatography System and controlled by Unicorn 6.3 software.
3. **Summary of Method**
   3.1. Method writing (programming)
   3.2. Manual control of the instrument
   3.3. Equilibration of system and column
   3.4. Fraction collector setup
   3.5. Application of sample
   3.6. Washing and elution of column
   3.7. Regeneration of system in preparation for subsequent run
   3.8. Procedures for cleaning and short or long term storage of the system
4. **References**
   4.1. Unicorn 6.3 Users Guide (electronic)
   4.2. AKTA pure 25 Users Guide (electronic)
   4.3. Manufacturer's literature/spec sheets for media
      4.3.1. HiTrap SP HP 5 information booklet (GE)
      4.3.2. L-Lysine HyperD reference sheet (Pall)
      4.3.3. Capto Q information booklet (GE)
      4.3.4. Ni^{2+} IMAC Profinity reference sheet (BioRad)
   4.4. End-of-Run t-PA Process SOP: Harvest, Centrifugation, Concentration, pH Adjustment (Document Number DP 3); 13Apr15
5. **Definitions**
   5.1. N/A
6. **Precautions**
   6.1. Routine care should be exercised in handling of buffers and samples of biological materials, which may have harmful biological activity in the case of accidental ingestion, needle stick, etc.
   6.2. User should read and be familiar with general good practice as outlined in the AKTA pure Cue Cards located near the instrument.
   6.3. Avoid damaging the threads through the use of excessive force when connecting plastic fasteners.
   6.4. Care must be taken to avoid air in the fluid path, which could damage the pumps or give spurious and uninterpretable readout from the UV and/or conductivity detectors.

Equipment SOP: Operation of ÄKTA pure Chromatography System

6.5. Gloves and protective eyewear should be worn when handling samples and reagents (buffers), however it is preferable that the user remove gloves prior to entering commands via the computer keyboard or mouse.

6.6. Buffers must be degassed and filtered prior to use with the AKTA pure instrument. Samples should be centrifuged at 10000x*g* for 5 min before injection/introduction into the fluid path.

6.7. Equipment calibration check: The AKTA pure system calibration is automatic; baseline for measurements of A280 and conductivity are zeroed at the beginning of a chromatography run. Further adjustment is beyond the scope of this document and should be referred to a qualified technician.

7. Responsibilities

7.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.

7.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

8. Equipment and Materials

8.1. AKTA pure chromatography system

8.2. Additional Lab Equipment: pH meter, balance

8.3. Lab Utensils: Beakers (250, 500ml), 500 ml graduated cylinders

8.4. Reagents: Filtered deionized water (MilliQ or similar), 20% ethanol

8.5. Lab Supplies: Filters (0.2 μm) and bottles for vacuum filtration and degassing of all chromatography buffers. Syringe (1ml). Tubes for fraction collector (3 ml – 15 ml capacity).

8.6. Column cleaning solution for HiTrap SP (GE) – 0.1M NaOH

8.7. Column cleaning solution for Lysine HyperD (Pall) – 0.1M NaOH;

8.8. Column cleaning solution for Capto-Q (GE) – 0.1M NaOH

8.9. Column cleaning solution for Ni2+ IMAC Profinity (Bio Rad) -

9. Procedure

9.1. ***Sample Collection and Preparation*** is described in "End-of-Run t-PA Process SOP: Harvest, Centrifugation, Concentration, pH Adjustment" Please refer to that document (DP 3, 13Apr15). The operator will require 0.6 ml of sample per sample injection (see below).The goal (analytical vs preparative) of the chromatography will dictate the amount of sample needed and/or applied to the column. Concentration of the sample and buffer components/pH should be chosen to be compatible with the mode of separation.

9.2. ***Reagent Recommendations***: Buffers should be prepared from the highest available grade reagents and water, filtered through a 0.2 μm filter and degassed thoroughly. Buffer preparation is beyond the scope of this document. Examples of buffers for specific applications with t-PA are :

9.2.1. HiTrap SP HP Cation exchange column:

9.2.1.1.Buffer A = 0.2M sodium acetate, pH 5.0, 0.01% Tween 80

9.2.1.2.Buffer B: 0.2M sodium acetate, pH 5.0, 0.01% Tween 80, 1M NaCl

9.2.2 L-Lysine Ceramic HyperD Affinity column:

Buffer A: 20mM sodium phosphate, pH 7.5, 0.01% Tween 80

Equipment SOP: Operation of ÄKTA pure Chromatography System

Buffer B: 20mM sodium phosphate, pH 7.5, 0.01% Tween 80, 1M NaCl
Elution Buffer: 20mM sodium phosphate, pH 7.5, 0.01% Tween 80, 0.2M arginine hydrochloride

9.2.3 Ni^{2+}-IMAC column:
Buffer A: 20mM sodium phosphate, pH 7.0, 0.5M NaCl 0.01% Tween 80
Buffer B: 20mM sodium phosphate, pH 7.0, 0.5M NaCl, 0.01% Tween 80, 0.5M imidazole

9.3. ***Method writing in Unicorn 6.3***.

For the Unicorn 6.3 software to be able to control the AKTA pure instrument, it is necessary to assemble programmed steps into a method. The steps vary for a given column and protein of interest, but typically include Sample Loading, Column Washing, Elution, and Equilibration. Often more steps are required. In most situations, the method will be written and stored in the Method Navigator within Method Editor; refer to the Batch Process Record or Process SOP for the protein of interest to find the name of the specific method. In the event a new or modified method is required, here are the (minimum) steps needed.

9.3.1. In the Unicorn software, navigate to the Method Editor window.
9.3.2. Under the File menu, choose New Method.
9.3.3. In the pop-up window, choose a pre-defined method for the type of chromatography you will be doing, then click OK.
9.3.4. Each step in the program is referred to as a 'Phase' in the software. The phases for the predefined method will appear in the center pane of the Method Editor window.
9.3.5. Click the Method Settings phase to display its properties in the right hand pane. If you are using a GE Life Sciences prepacked column, find and select it in the Column Type dropdown menu. Its properties and suggested flow rate will automatically be chosen. If your column is not listed, enter the Column Volume, Pressure Limit and Flow Rate (specified by the manufacturer) in their respective boxes. Choose Inlets, usually A1 and B1; these can be specified for each step (phase) as you progress through method writing.
9.3.6. While still in the Method Settings pane, click the Start Protocol button. A pop-up window appears that allows the operator to choose items that will be presented for operator's information/approval when a run of the method is initiated. Check the boxes for Method Information (which will show the expected volume and time required, allowing one to check that the pumps will not run dry due to inadequate supply of buffer), and for Questions. Click the button labeled Define Questions. This allows the operator to specify useful information that must be entered before the run can start, which may include sample characteristics, buffer composition/preparation date, operator, etc. Follow the prompts to enter questions, check the Mandatory and Display in Chromatogram boxes, then Preview to confirm the appearance.
9.3.7. Click the button for Equilibration phase in the center pane to display its properties in the right pane. Confirm that 'Reset UV monitor' is checked. Check 'Fill the system with the selected buffer' to quickly flush inappropriate buffers between the pumps and the injection valve.

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9.3.8. Click the Sample Application phase in the center pane to display the parameters.
- 9.3.8.1. Uncheck the box 'Use the same flow rate as in Method Settings' – typically a capture method such as ion exchange or affinity separations achieves higher binding efficiency at lower flow rates.
- 9.3.8.2. Select the Loop type, and enter the sample volume to be injected. In the 'Empty loop with' box, enter a volume sufficient to flush all sample from the loop.
- 9.3.8.3. Confirm that the correct inlet lines are specified; most methods use A1 and B1.
- 9.3.8.4. If you suspect that your sample will exceed the capacity of the column, check the 'Interrupt sample application at UV' and enter a value (which will likely have to be determined empirically). A better approach is to limit the quantity of sample applied so that the operator is confident that the capacity of the column will be adequate to the task.
- 9.3.8.5. In the 'Fractionate' section, choose the path that the eluent should follow. It is customary to collect the unbound protein in a container ('using outlet valve' directs the flow-thru to the tube labeled 'Out'; place it in a clean flask) or in fractions ('using fraction collector').
- 9.3.8.6. Fractionation settings: choose 'Fixed outlet' using outlet valve. Choose 'fixed volume fractionation' to collect the flow-thru in constant volume fractions with the fraction collector.

9.3.9. Click the Column Wash phase button in the center pane.
- 9.3.9.1. Check the box to 'Use the same flow rate as in Method Settings'.
- 9.3.9.2. Confirm the inlets for A and B, and that the 'Fill the system….' box is unchecked.
- 9.3.9.3. Select a value for 'Wash until', which should be at least 10 column volumes (for the small columns used in this course). Select the radio button for 'the following condition is met', choose Stable UV, set the Accepted UV fluctuation to 0.1mAU (experience may dictate a change, so revisit this setting on occasion), and set the Maximum wash volume to 20 CV.
- 9.3.9.4. Fractionate: column wash is diverted 'in waste (do not collect)'.

9.3.10. Click the Elution button in the center pane and make these settings:
- 9.3.10.1. Check the box for 'Use the same flow rate as in Method Settings'
- 9.3.10.2. Confirm Inlet A and B specify the tubing that is placed in buffers A and B.
- 9.3.10.3. Gradient elution is the default selection.
  - 9.3.10.3.1. Choose the 'Start at' concentration of buffer B, usually 0%. If greater than 0%, check the 'Fill the system….' box.
  - 9.3.10.3.2. Specify the Type of gradient (step or linear), target % B and total volume (recommend 10 CV initially) for the gradient in this segment.
  - 9.3.10.3.3. Add more segments as needed. Commonly a final elution of 2-3 CV at high strength eluting conditions (e.g. 1M NaCl for an ion exchange separation) is included.

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9.3.10.3.4. Fractionate settings should be for 'using fraction collector' and 'fixed volume fractionation'. Set the Fixed fractionation volume to a volume that represents about 1/20 of the total gradient volume.

9.3.11. Click the (second) Equilibration button to set parameters for re-equilibration of the column for subsequent runs.

9.3.11.1. Confirm that the 'Reset UV Monitor' box is unchecked.

9.3.11.2. Check the 'Use the same flow rate…..' box.

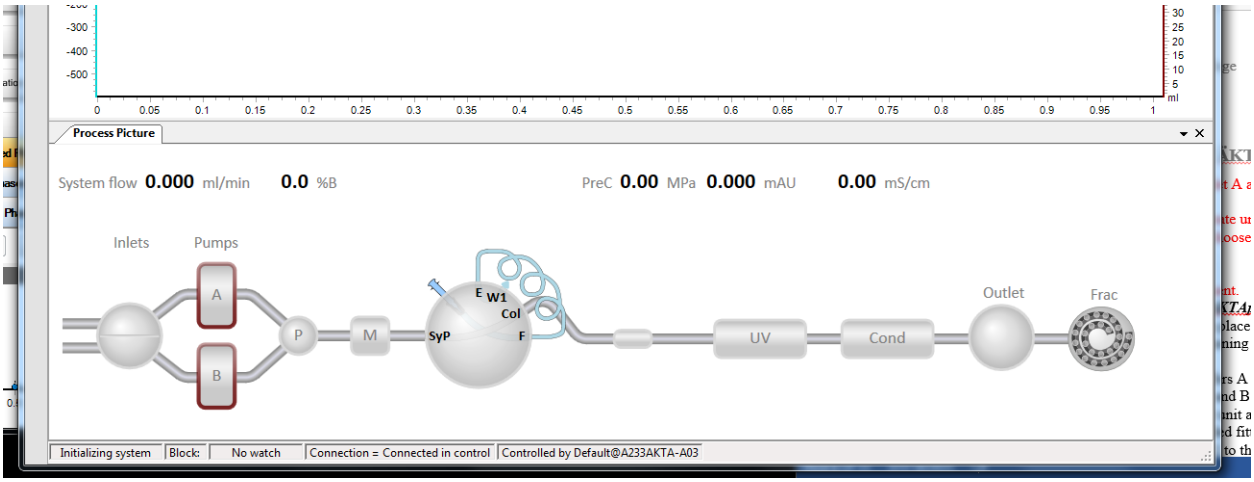
9.3.11.3. Confirm that Inlet A and B are correct and correspond to the buffers A and B.

9.3.11.4. Set the 'Equilibrate until' volume to 5 CV.

9.3.12. Under the File menu, choose Save and name the method uniquely; select the correct folder and Save.

9.4. ***Manual Control of the instrument.***

The AKTA pure instrument may be controlled 'manually' via the System Control window lower pane graphic interface (or 'Process Picture'), as pictured here.



9.4.1. A control panel that allows selection of inlet pumps/lines A1, A2 B1 and B2 will appear when one clicks on the small bisected circle on the left side.

9.4.2. By clicking on either Pump A or Pump B in the depiction, you can access a dialog box which allows for quick setting of flow rate and buffer composition, as well as convenient pump wash features as shown:

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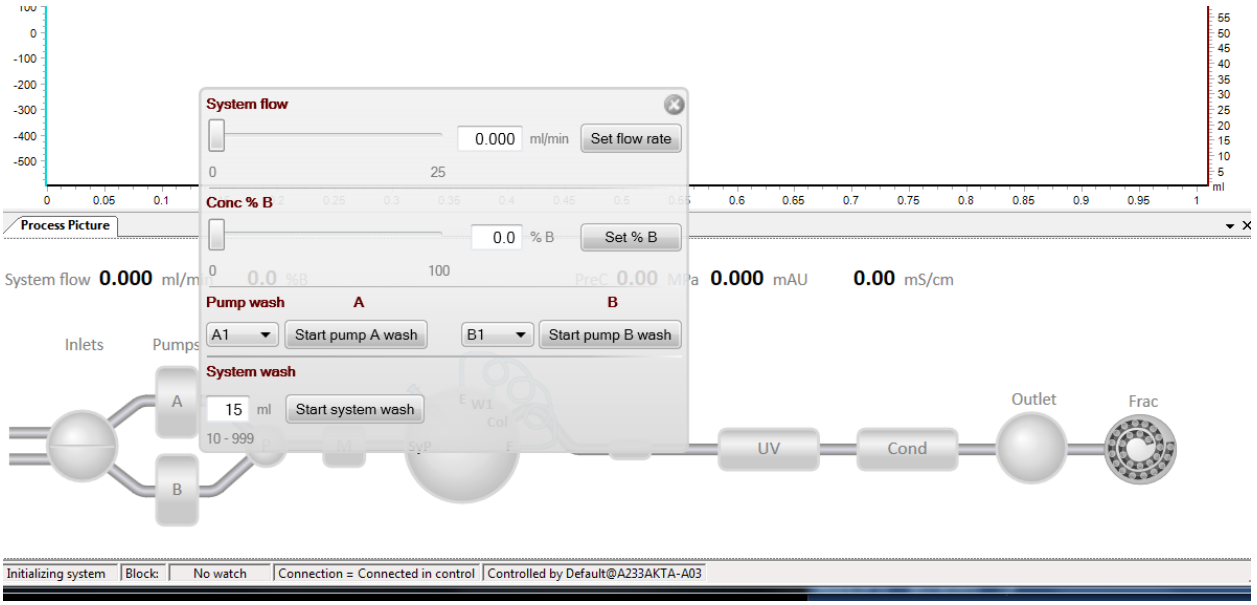
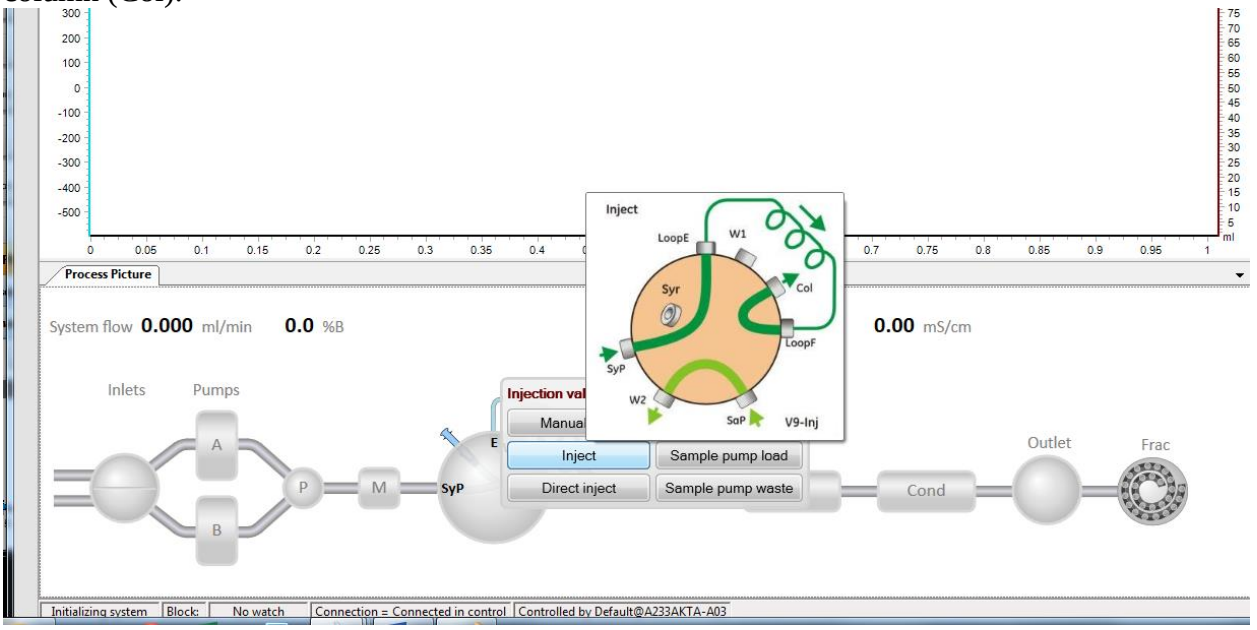


9.4.3. The large circle (approximately the center of components) in the diagram represents the sample injection valve. Upon clicking it, one will see a panel of 6 options for the position of the valve (five are visible in the diagram below). Hovering over each option causes a new diagram to appear that shows the flow path through the valve for that particular selection; shown here is the Inject position, with flow coming from the system pumps (SyP), going through LoopE valve inlet, carrying contents of the sample loop out through LoopF valve outlet and onto the column (Col).



Equipment SOP: Operation of ÄKTA pure Chromatography System

9.4.4. Clicking the UV detector icon reveals a command to 'Auto Zero UV'.
9.4.5. The small circle to the right is the Outlet valve. By clicking on it with the electronic mouse pointing device, the operator may select whether the eluent goes to Waste, the Outlet tubing, or the Fraction Collector.
9.4.6. Various parameters of the current run condition are displayed directly above the manual control interface. Pay particular attention to pre-column pressure (PreC x.xx MPa) to avoid over pressuring/damaging the column. Most of our columns have a limit of 0.5 MPa.

9.5. ***Start-up and preparation of AKTA pure Instrument and computer***:
Degassed buffers should be in place prior to turning on the AKTA pure instrument. Equipment start-up requires turning on the instrument and, separately, the computer connected to it.
9.5.1. Place the degassed buffers A and B on top of the AKTA pure instrument.
9.5.2. Locate Inlet tubing A1 and B1 (atop the instrument and resting in water or 20% ethanol). Each has a filter unit attached, which distinguishes them from A2 and B2; those end in a male threaded fitting and will not be used for a two-buffer procedure.
9.5.3. Transfer tubing Inlet A1 to the buffer A bottle.
9.5.4. Transfer tubing Inlet B1 to the buffer B bottle.
9.5.5. The On/Off switch for the instrument is located on the right side toward the rear of the housing. Switch to the 'On' position. Audible emanations from within the instrument cabinet indicate that the AKTA pure system is going through its brief initialization sequence.
9.5.6. The computer On/Off switch is located on the front of the Dell desktop computer unit, near the top of the case. Press in to turn on the computer.
9.5.7. Login to the computer using credentials provided by the College.
9.5.8. Double click the Unicorn 6.3 icon on the desktop to open the software which controls the instrument functions. Click OK in the "Log In – Unicorn" dialog box that appears.
9.5.9. Open the System Control window (under Tools menu, if not opened automatically on startup).
9.5.10. The top pane of the window will show the current state of the instrument, and the bottom pane shows the fluid path and manual controls. If the window is blank, go to the System menu and select Connect to Systems, check the box by AKTA pure 25 and click OK.
9.5.11. Confirm that the correct column (HiTrap SP 5 ml) is attached to the system. If not, refer to Section 9.4 (Installing/Changing a Chromatography Column on the AKTA pure Chromatography System).
9.5.12. Under the File menu, choose Open and select the method with file name "HiTrap SP 5ml Equilibration".
9.5.13. A dialog box appears that allows the method to be run. Click Start to initiate flushing of the pumps and equilibration of the column.
9.5.14. While the equilibration method is running, prepare the fraction collector for later steps by filling the carousel with clean tubes.

Equipment SOP: Operation of ÄKTA pure Chromatography System

9.5.15. Allow the program to run to completion, about 15 minutes.

9.6. ***Installing/Changing a Chromatography Column on the AKTA pure Chromatography System.***

It is imperative that the following operations be performed in such a way as to prevent the introduction of air bubbles into the column and fluid path, which is achieved by making liquid-to-liquid (drop-to-drop) contact prior to inserting the threaded fitting into its position.

9.6.1. Have on hand a few paper lab towels and a 250 ml beaker to catch waste.
9.6.2. Remove tube connector from the UV detector inlet by unscrewing the knurled fastener.
9.6.3. Initiate flow manually at 0.5 ml/min collecting waste in the beaker or towel.
9.6.4. Remove the plug from the column inlet and place a few drops of 20% ethanol in the inlet, filling it to insure the absence of air. Also add drops of 20% ethanol to the UV detector inlet.
9.6.5. As a droplet emerges from the inlet tubing, touch it to the liquid in the column inlet and begin to thread the fitting in, leaving slight looseness of threads so that liquid escapes around the fitting and pressure buildup in the column is prevented.
9.6.6. Remove the column bottom plug and screw the column directly into the UV detector inlet.
9.6.7. Tighten the column inlet fitting just enough to prevent leaking.
9.6.8. The column is now ready to equilibrate in buffer (step 9.3.12) prior to performing a chromatography run.

9.7. ***How to fill a sample loop.***

9.7.1. Fill an appropriately sized syringe with sample, taking care to remove bubbles.
9.7.2. Connect the syringe to the injection port (see Fig. 4).
9.7.3. Open the System Control module in the Unicorn software.
9.7.4. In the Process Picture click the Injection Valve and select Manual load to confirm that the valve is in, or switch the valve to, manual load position.
9.7.5. Depress the syringe plunger to inject sample into the sample loop, and leave the syringe attached to the port (prevents sample loss due to siphoning). Regarding amount to inject, if sample preservation is a consideration, inject only enough to fill the loop. If sample is abundant and/or relatively easy to generate, inject about twice the loop volume to insure complete filling.

9.8. ***Performing a chromatography run***:

9.8.1. Place the fraction collector tube 1 near the outlet tubing from the instrument (refer to attachment Fig 1) so that it will touch the arrow on the white paddle of the fraction collector arm. Note: To rotate the carousel, reach around the left side of the collector to find a rubber roller pressing against the carousel (Fig 2). Pull the roller away from the carousel (Fig. 3); the carousel will rotate freely as long as the roller is held. When the first tube is in the correct position, release the roller.
9.8.2. Gently raise the arm and swing it into position against tube 1.
9.8.3. Place all ‘Waste’ tubing, labeled W, W1 & W2 in 1 L Erlenmeyer flask.
9.8.4. Place the tube labeled Out in a 125 ml Erlenmeyer flask.

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9.8.5. Using a 1 ml syringe, aspirate 0.6 ml of the tPA sample into the syringe, expel any bubbles and insert the loaded syringe into the injection port.
9.8.6. Inject the sample into the port to fill the 0.5 ml sample loop.
9.8.7. Open the Unicorn software and navigate to the System Control window.
9.8.8. Under the File menu, choose Open and select the method with file name "HiTrap SP tPA Cation Exchange".
9.8.9. In the dialog box that opens, enter operator's name, sample notes.
9.8.10. Click Next; note the time and volume for the run; make sure there is excess buffer A and B.
9.8.11. Click Next. Record the buffer composition of each buffer and the sample identity.
9.8.12. Click Next. Enter a filename composed of the method name, date, operator or group initials, for example "HiTrapSP tPA 16May15 CertGroup".
9.8.13. Click Start. The instrument should begin to execute the method, as evidenced by a soothing hum from the pumps and drops of liquid falling into tube 1 from the fraction collector outlet.
9.8.14. Observe that the fraction collector is receiving drops.
9.8.15. Monitor the computer screen for error messages or warnings.
9.8.16. Allow the method to run to completion, at which time the system will be re-equilibrated and ready for subsequent runs by repeating section 9.4.

9.9. ***Equipment shut-down and short term (less than 3 days) storage***

9.9.1. After completion of the final separation of the day, transfer Inlet tubing A1 and B1 to a flask of degassed MilliQ water (250ml or greater).
9.9.2. In the Unicorn software, open the System Control window.
9.9.3. Under the File menu, choose Open, then select the method 'System Short Term Storage'.
9.9.4. Click Start.
9.9.5. Allow the method to run to completion, as indicated by an audible tone and onscreen window.
9.9.6. Turn off the instrument or perform the long term storage routine as needed (section 9.6).

9.10. ***Equipment shut-down and long term (3 days or more) storage***

9.10.1. After completion of the System Short Term Storage method, transfer Inlet tubing A1 and B1 to a flask of degassed 20% ethanol (250ml or greater).
9.10.2. In the Unicorn software, open the System Control window.
9.10.3. Under the File menu, choose Open, then select the method 'System Long Term Storage'.
9.10.4. Click Start.
9.10.5. Allow the method to run to completion, as indicated by an audible tone and onscreen window.
9.10.6. Turn off the instrument.

Equipment SOP: Operation of ÄKTA pure Chromatography System

9.11. ***Cleaning the system***

9.11.1. Column Removal – have paper towels on hand to catch drips. You will also need an appropriate plug for each end of the column.

9.11.1.1. Use the Manual Control in the System Control window to set the flow rate of buffer or water to 0.5 ml/min.

9.11.1.2. Disconnect the column outlet from the UV detector and fit the connector with a plug, slowly so that emerging droplets displace *all* air from the plug. Leave the plug slightly loose so as to prevent backpressure buildup.

9.11.1.3. Slowly disconnect the tubing from the column inlet, allowing liquid to fill the cavity where the connector attaches. Attach the tubing directly to the UV detector inlet and stop the flow of buffer/water.

9.11.1.4. Completely tighten the column outlet plug, but be careful not to overtighten and strip the threads.

9.11.1.5. Carefully insert a plug into the column inlet threads, displacing liquid but not allowing air to enter.

9.11.1.6. The column may now be stored.

9.11.2. Minimal Cleaning – After every run, perform short-term storage (9.10).

9.11.3. Thorough Cleaning – Should be performed weekly.

9.11.3.1. Remove the column (9.12.1) from the system prior to thorough cleaning of the system with 0.5M NaOH.

9.11.3.2. Immerse all pump inlet tubes in a container of 0.5M NaOH.

9.11.3.3. Run the method ‘System Clean’.

9.11.3.4. Remove the pump inlet tubing from the NaOH container, rinse each carefully with a squirt bottle of MilliQ water and place in a flask of filtered and degassed MilliQ water.

9.11.3.5. Run the method ‘System Short Term Storage’.

9.12. ***Chromatogram printout***

9.12.1. In the Unicorn software interface, open the Evaluation window.

9.12.2. In the Result Navigator pane, click the Results tab.

9.12.3. Locate the file of interest and double click its name to display your chromatogram in the right pane.

9.12.4. *Optional:* Click the Customize button to open a dialog box that allows you to specify what curves display and the scale of each axis. Recommended are the UV Chrom curve, Conductivity, and Fraction Number.

9.12.5. Click the Report button, check the Default report in the selection window and click Preview.

9.12.6. Under File, choose to Print (or Save as PDF to use a different printer).

Equipment SOP: Operation of ÄKTA pure Chromatography System

10. Attachments/Figures

Fig. 1. ***Diagram of AKTA pure instrument, fraction collector and computer***

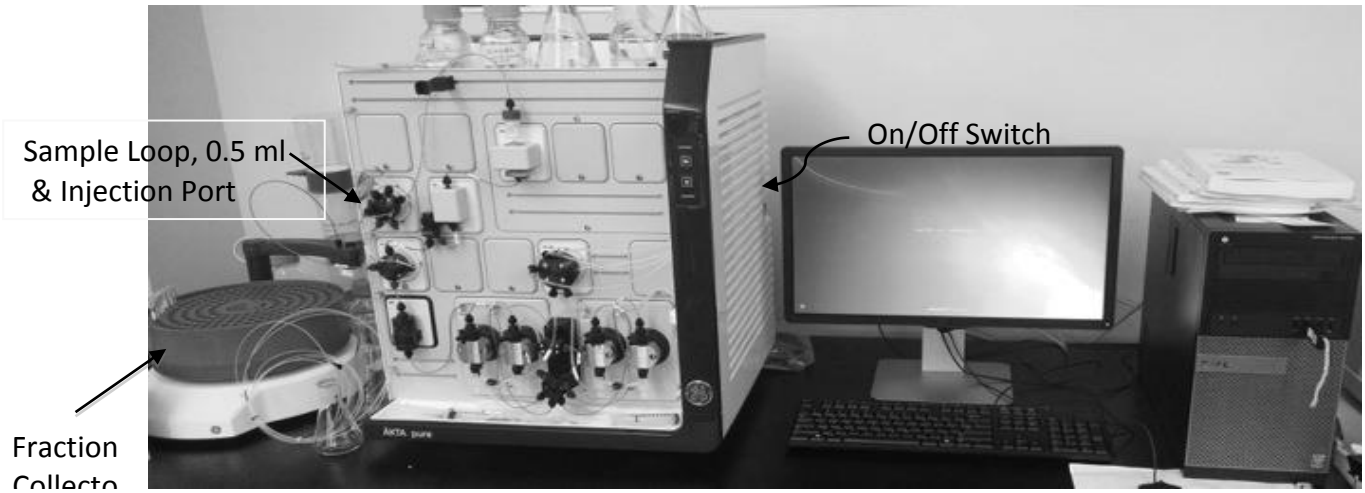
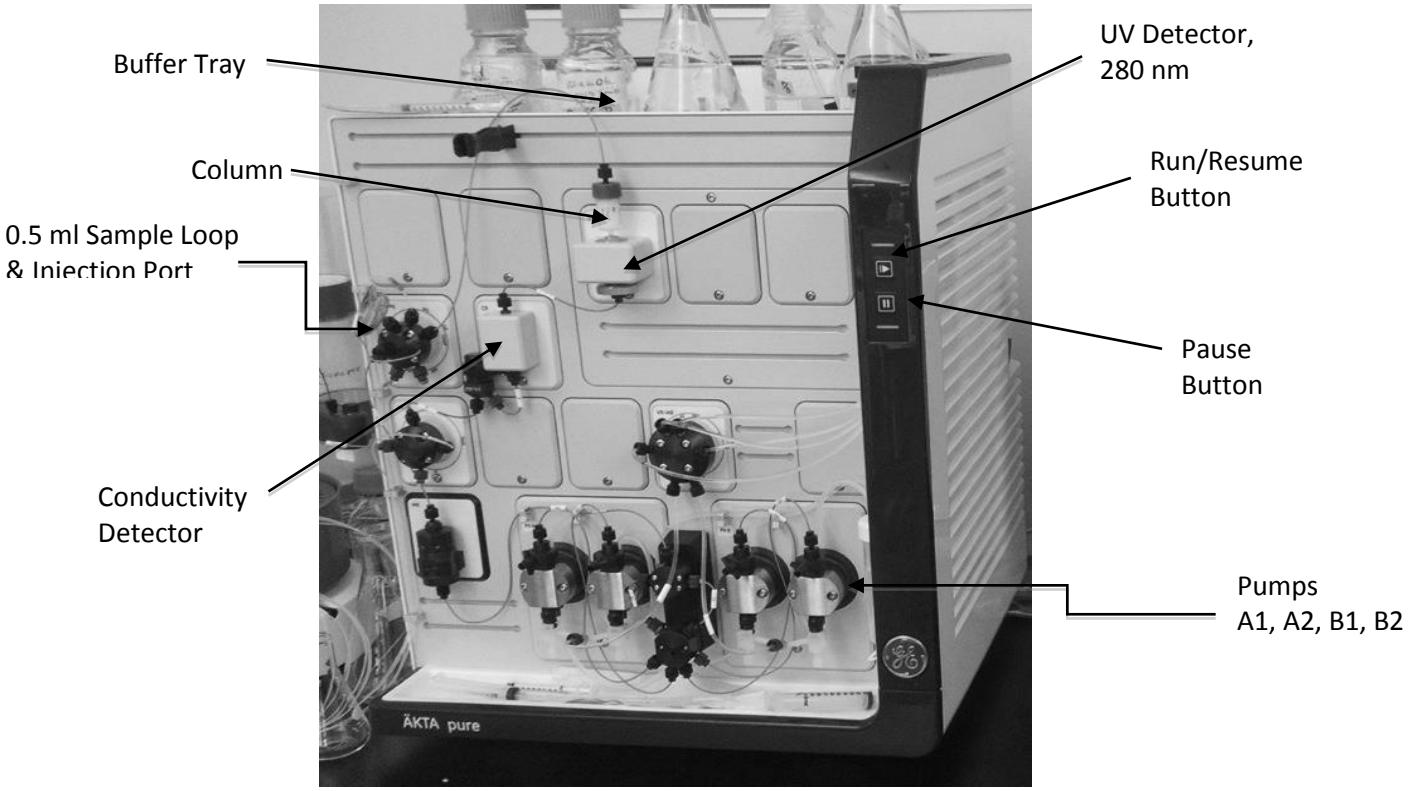


Fig. 2. ***AKTA pure Instrument Features***



Equipment SOP: Operation of ÄKTA pure Chromatography System

Fig 3. ***System Control window within the Unicorn 6.3 software.***

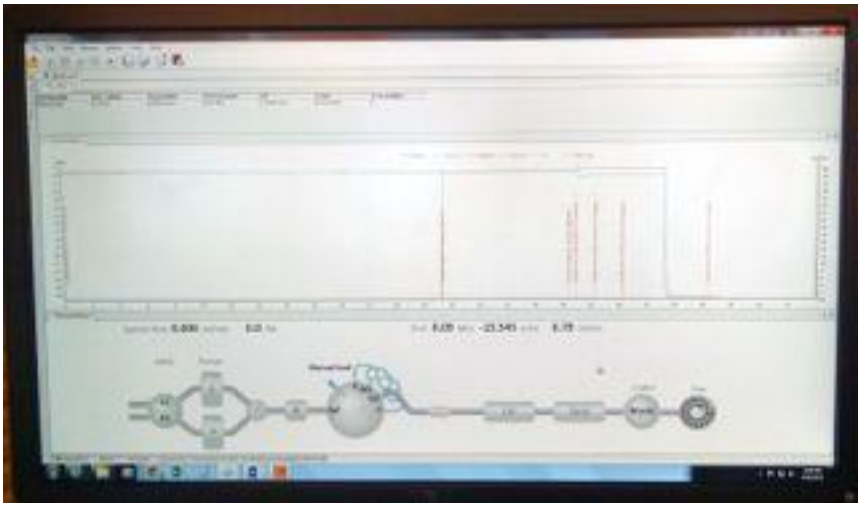
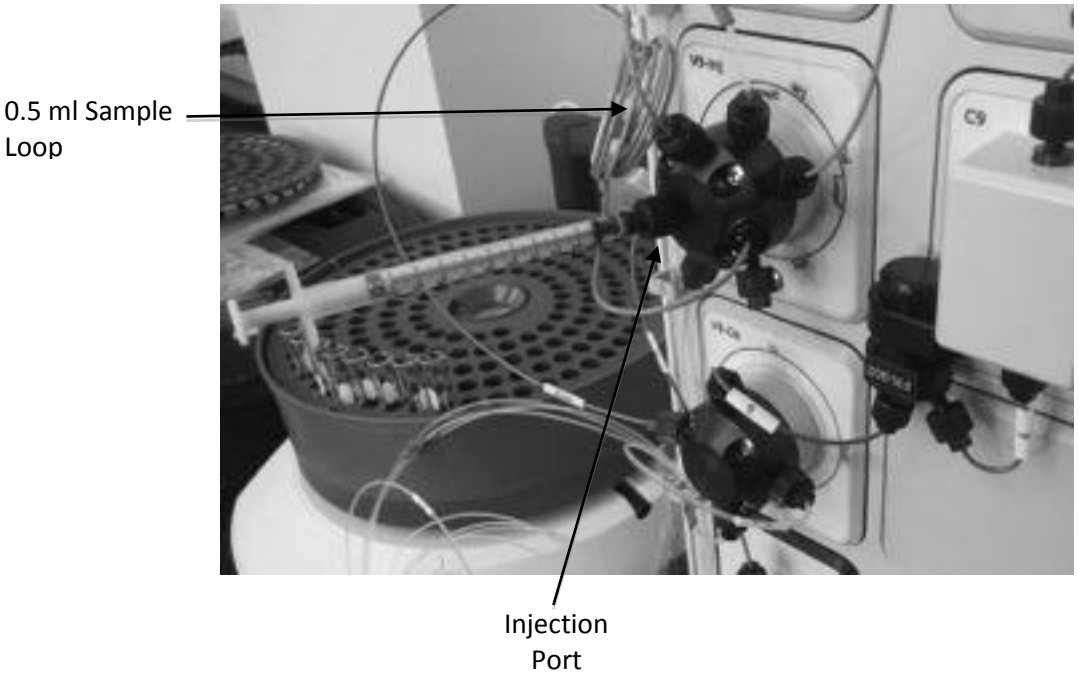


Fig. 4. ***Detail of Injection Port with Syringe in Place***.



Equipment SOP: Operation of ÄKTA pure Chromatography System

Fig. 5. ***Fraction collector carousel rubber advancement roller/gear.***

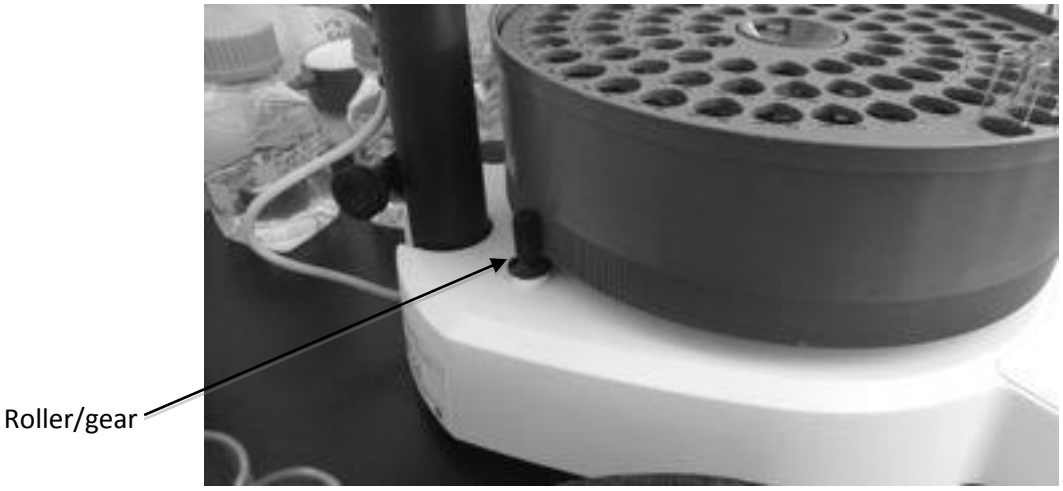
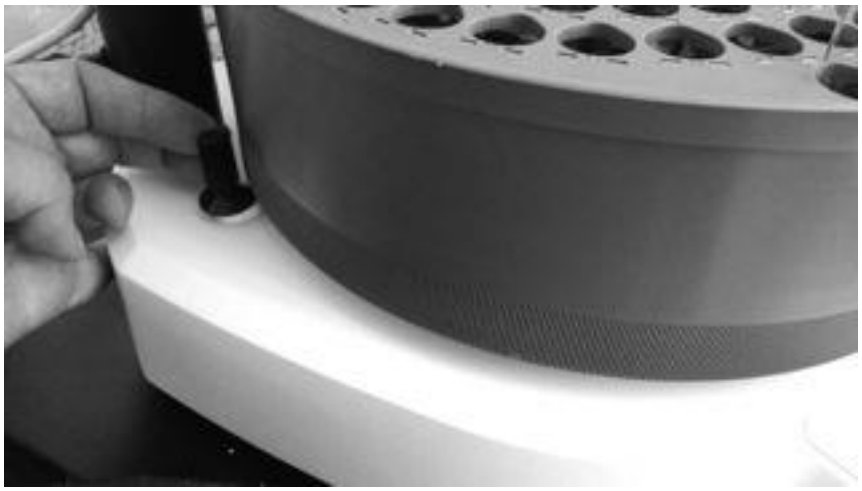


Fig. 6. ***Release of roller to allow free rotation of the carousel.***



Equipment SOP: Operation of ÄKTA pure Chromatography System

Fig. 7. ***Location of tube #1 under the fraction collector drip outlet.***

11. History

Revision Number	*Effective Date*	*Preparer*	*Description of Change*
0	17JUL15	Dr. David Frank	Initial release

SOP: Operation of ÄKTA pure Chromatography System for Cation Exchange Chromatography of t-PA

Approvals

Preparer:	Dr. David Frank	Date: 15JUL15
Reviewer:	Jason McMillan	Date: 16JUL15
Reviewer:	Dr. Maggie Bryans	Date: 16JUL15

1. **Purpose**
   1.1. This procedure describes the operation of the ÄKTA pure Chromatography System, controlled by Unicorn 6.3 software, for the purpose of cation exchange chromatography of samples containing tissue type plasminogen activator (tPA).
2. **Scope and Applicability**
   2.1. Applies to purification of t-PA from prepared conditioned medium, which has been concentrated and its pH adjusted to 4.5, using a HiTrap SP HP 5 ml column installed on the GE ÄKTA pure Chromatography System and controlled by Unicorn 6.3 software.
3. **Summary of Method**
   3.1. Preparation of buffer(s)
   3.2. Equilibration of system and column
   3.3. Fraction collector setup
   3.4. Application of sample
   3.5. Washing and elution of column
   3.6. Regeneration of system in preparation for subsequent run
   3.7. Procedures for short or long term storage of the system
4. **References**
   4.1. Unicorn 6.3 Users Guide (electronic)
   4.2. AKTA pure 25 Users Guide (electronic)
   4.3. HiTrap SP HP 5 information booklet
   4.4. End-of-Run t-PA Process SOP: Harvest, Centrifugation, Concentration, pH Adjustment (Document Number DP 3); 13Apr15
5. **Definitions**
   5.1. N/A
6. **Precautions**
   6.1. Routine care should be exercised in handling of buffers and samples of biological materials, which may have harmful biological activity in the case of accidental ingestion, needle stick, etc.
   6.2. User should read and be familiar with general good practice as outlined in the AKTA pure Cue Cards located near the instrument.
   6.3. Avoid damaging the threads through the use of excessive force when connecting plastic fasteners.
   6.4. Care must be taken to avoid air in the fluid path, which could damage the pumps or give spurious and uninterpretable readout from the UV and/or conductivity detectors.

SOP: Operation of ÄKTA pure Chromatography System for Cation Exchange Chromatography of t-PA

6.5. Gloves and protective eyewear should be worn when handling samples and reagents (buffers), however it is preferable that the user remove gloves prior to entering commands via the computer keyboard or mouse.

6.6. Buffers must be degassed and filtered prior to use with the AKTA pure instrument. Samples should be centrifuged at 10000x*g* for 5 min before injection/introduction into the fluid path.

6.7. Equipment calibration check: The AKTA pure system calibration is automatic; baseline for measurements of A280 and conductivity are zeroed at the beginning of a chromatography run. Further adjustment is beyond the scope of this document and should be referred to a qualified technician.

7. Responsibilities

7.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.

7.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

8. Equipment and Materials

8.1. AKTA pure chromatography system

8.2. Additional Lab Equipment: pH meter, balance

8.3. Lab Utensils: Beakers (250, 500ml), 500 ml graduated cylinders

8.4. Reagents: Glacial acetic acid, sodium chloride, filtered deionized water (MilliQ or similar). 10 % w/v Tween 80, 20% ethanol, NaOH

8.5. Lab Supplies: Filters (0.2 μm) and bottles for vacuum filtration and degassing of all chromatography buffers. Syringe (1ml). Tubes for fraction collector.

9. Procedure

9.1. ***Sample Collection and Preparation*** is described in "End-of-Run t-PA Process SOP: Harvest, Centrifugation, Concentration, pH Adjustment" Please refer to that document (DP 3, 13Apr15). The operator will require 0.6 ml of sample per sample injection (see below).

9.2. ***Reagent Preparation***: Buffers should be prepared dependent on the mode of separation employed; in this instance cation exchange chromatography provides good separation of tPA from contaminating protein at pH 5.0.

9.2.1. Cation Exchange Buffer: 0.2M Sodium Acetate, pH 5.0, 0.1% Tween 80

9.2.1.1. Dissolve 11.5 ml glacial Acetic Acid in 950 ml filtered deionized water in a one liter beaker, with stir bar.

9.2.1.2.. Prepare 10M NaOH by dissolving 40.0 gm solid in filtered deionized water. Adjust the final volume to 100 ml. Use CAUTION as the solution becomes very hot, and is caustic.

9.2.1.3. Titrate the pH of the HOAc solution to 5.0 by addition of 10M NaOH. Use a 3 ml transfer pipet to add about 15 ml to the HOAc, then proceed dropwise until pH 5.0 is obtained.

SOP: Operation of ÄKTA pure Chromatography System for Cation Exchange Chromatography of t-PA

9.2.1.4. Add 10 ml of 10% Tween 80, then adjust the final volume to 1000 ml. Set aside 500 ml of this solution and label 'Buffer A' along with its precise composition and date of preparation. Filter and degas the buffer by passage through a vacuum filter device attached to house vacuum, leaving the filtered solution under vacuum for 15-20 minutes.

9.2.1.5. Use the remaining 500 ml to dissolve 29.22 gm of NaCl in a 400 ml beaker. Following dissolution, filter and degas this mixture and label the bottle 'Buffer B', along with the actual contents (0.2 M NaOAc, pH 4.5, 0.1% Tween80, 1 M NaCl).

9.3. ***Start-up and preparation of AKTA pure Instrument and computer***:

Degassed buffers should be in place prior to turning on the AKTA pure instrument. Equipment start-up requires turning on the instrument and, separately, the computer connected to it.

9.3.1. Place the degassed buffers A and B on top of the AKTA pure instrument.

9.3.2. Locate Inlet tubing A1 and B1 (atop the instrument and resting in water or 20% ethanol). Each has a filter unit attached, which distinguishes them from A2 and B2; those end in a male threaded fitting and will not be used for this procedure.

9.3.3. Transfer tubing Inlet A1 to the buffer A bottle.

9.3.4. Transfer tubing Inlet B1 to the buffer B bottle.

9.3.5. The On/Off switch for the instrument is located on the right side toward the rear of the housing. Switch to the 'On' position. Audible emanations from within the instrument cabinet indicate that the AKTA pure system is going through its brief initialization sequence.

9.3.6. The computer On/Off switch is located on the front of the Dell desktop computer unit, near the top of the case. Press in to turn on the computer.

9.3.7. Login to the computer using credentials provided by the College.

9.3.8. Double click the Unicorn 6.3 icon on the desktop to open the software which controls the instrument functions. Click OK in the "Log In – Unicorn" dialog box that appears.

9.3.9. Open the System Control window (under Tools menu, if not opened automatically on startup).

9.3.10. The top pane of the window will show the current state of the instrument, and the bottom pane shows the fluid path and manual controls. If the window is blank, go to the System menu and select Connect to Systems, check the box by AKTA pure 25 and click OK.

9.3.11. Confirm that the correct column (HiTrap SP 5 ml) is attached to the system. If not, refer to Section 9.4 (Installing/Changing a Chromatography Column on the AKTA pure Chromatography System).

9.3.12. Under the File menu, choose Open and select the method with file name "HiTrap SP 5ml Equilibration".

9.3.13. A dialog box appears that allows the method to be run. Click Start to initiate flushing of the pumps and equilibration of the column.

SOP: Operation of ÄKTA pure Chromatography System for Cation Exchange Chromatography of t-PA

9.3.14. While the equilibration method is running, prepare the fraction collector for later steps by filling the carousel with clean tubes.

9.3.15. Allow the program to run to completion, about 15 minutes.

9.4. ***Installing/Changing a Chromatography Column on the AKTA pure Chromatography System.***

It is imperative that the following operations be performed in such a way as to prevent the introduction of air bubbles into the column, which is achieved by making liquid-to-liquid (drop-to-drop) contact prior to inserting the threaded fitting into its position.

9.4.1. Have on hand a few paper lab towels and a 250 ml beaker to catch waste.

9.4.2. Remove tube connector from the UV detector inlet by unscrewing the knurled fastener.

9.4.3. Initiate flow manually at 0.5 ml/min collecting waste in the beaker or towel.

9.4.4. Remove the plug from the column inlet and place a few drops of 20% ethanol in the inlet, filling it to insure the absence of air.

9.4.5. As a droplet emerges from the inlet tubing, touch it to the liquid in the column inlet and begin to thread the fitting in, leaving slight looseness of threads so that liquid escapes around the fitting and pressure buildup in the column is prevented.

9.4.6. Remove the column bottom plug and screw the column directly into the UV detector inlet.

9.4.7. Tighten the column inlet fitting just enough to prevent leaking.

9.4.8. The column is now ready to equilibrate in buffer (step 9.3.12) prior to performing a chromatography run.

Performing a chromatography run:

9.4.9. Place the fraction collector tube 1 near the outlet tubing from the instrument (refer to attachment Fig 1) so that it will touch the arrow on the white paddle of the fraction collector arm. Note: To rotate the carousel, reach around the left side of the collector to find a rubber roller pressing against the carousel (Fig 2). Pull the roller away from the carousel (Fig. 3); the carousel will rotate freely as long as the roller is held. When the first tube is in the correct position, release the roller.

9.4.10. Gently raise the arm and swing it into position against tube 1.

9.4.11. Place all 'Waste' tubing, labeled W, W1 & W2 in 1 L Erlenmeyer flask.

9.4.12. Place the tube labeled Outlet in a 125 ml Erlenmeyer flask.

9.4.13. Using a 1 ml syringe, aspirate 0.6 ml of the tPA sample into the syringe, expel any bubbles and insert the loaded syringe into the injection port.

9.4.14. Inject the sample into the port to fill the 0.5 ml sample loop.

9.4.15. Open the Unicorn software and navigate to the System Control window.

9.4.16. Under the File menu, choose Open and select the method with file name "*HiTrap SP tPA Production*".

9.4.17. In the dialog box that opens, enter operator's name, sample notes.

9.4.18. Click Next; note the time and and volume for the run; make sure there is excess buffer A and B.

9.4.19. Click Next. Record the buffer composition of each buffer and the sample identity.

SOP: Operation of ÄKTA pure Chromatography System for Cation Exchange Chromatography of t-PA

9.4.20. Click Next. Enter a filename composed of the method name, date, operator or group initials, for example HiTrapSP tPA CEX 16May15 CertGroup.
9.4.21. Click Start. The instrument should begin to execute the method, as evidenced by a soothing hum from the pumps and drops of liquid falling into tube 1 from the fraction collector outlet.
9.4.22. Observe that the fraction collector is receiving drops.
9.4.23. Monitor the computer screen for error messages or warnings.
9.4.24. Allow the method to run to completion, at which time the system will be re-equilibrated and ready for subsequent runs by repeating section 9.4.

9.5. ***Equipment shut-down and short term (less than 3 days) storage***

9.5.1. After completion of the final separation of the day, transfer Inlet tubing A1 and B1 to a flask of degassed MilliQ water (250ml or greater).
9.5.2. In the Unicorn software, open the System Control window.
9.5.3. Under the File menu, choose Open, then select the method 'System Short Term Storage'.
9.5.4. Click Start.
9.5.5. Allow the method to run to completion, as indicated by an audible tone and onscreen window.
9.5.6. Turn off the instrument or perform the long term storage routine as needed (section 9.6).

9.6. ***Equipment shut-down and long term (3 days or more) storage***

9.6.1. After completion of the System Short Term Storage method, transfer Inlet tubing A1 and B1 to a flask of degassed 20% ethanol (250 ml or greater).
9.6.2. In the Unicorn software, open the System Control window.
9.6.3. Under the File menu, choose Open, then select the method 'System Long Term Storage'.
9.6.4. Click Start.
9.6.5. Allow the method to run to completion, as indicated by an audible tone and onscreen window.
9.6.6. Turn off the instrument.

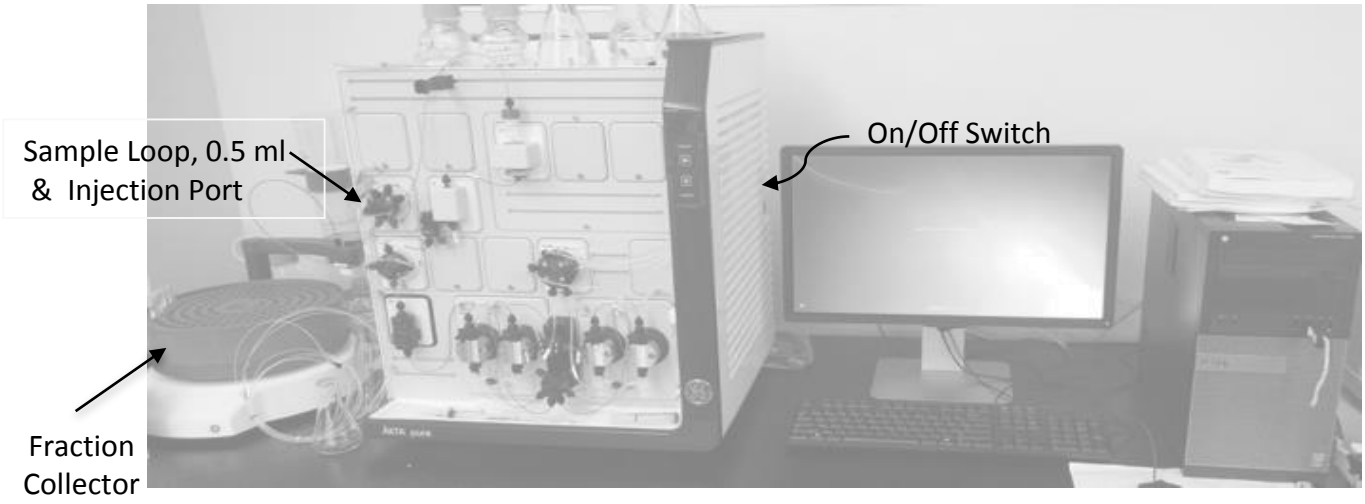
9.7. ***Chromatogram printout***

9.7.1. In the Unicorn software interface, open the Evaluation window.
9.7.2. In the Result Navigator pane, click the Results tab.
9.7.3. Locate the file of interest and double click its name to display your chromatogram in the right pane.
9.7.4. *Optional:* Click the Customize button to open a dialog box that allows you to specify what curves display and the scale of each axis. Recommended are the UV Chrom curve, Conductivity, and Fraction Number.
9.7.5. Click the Report button, check the Default report in the selection window and click Preview.
9.7.6. Under File, choose to Print (or Save as PDF to use a different printer).

SOP: Operation of ÄKTA pure Chromatography System for Cation Exchange Chromatography of t-PA

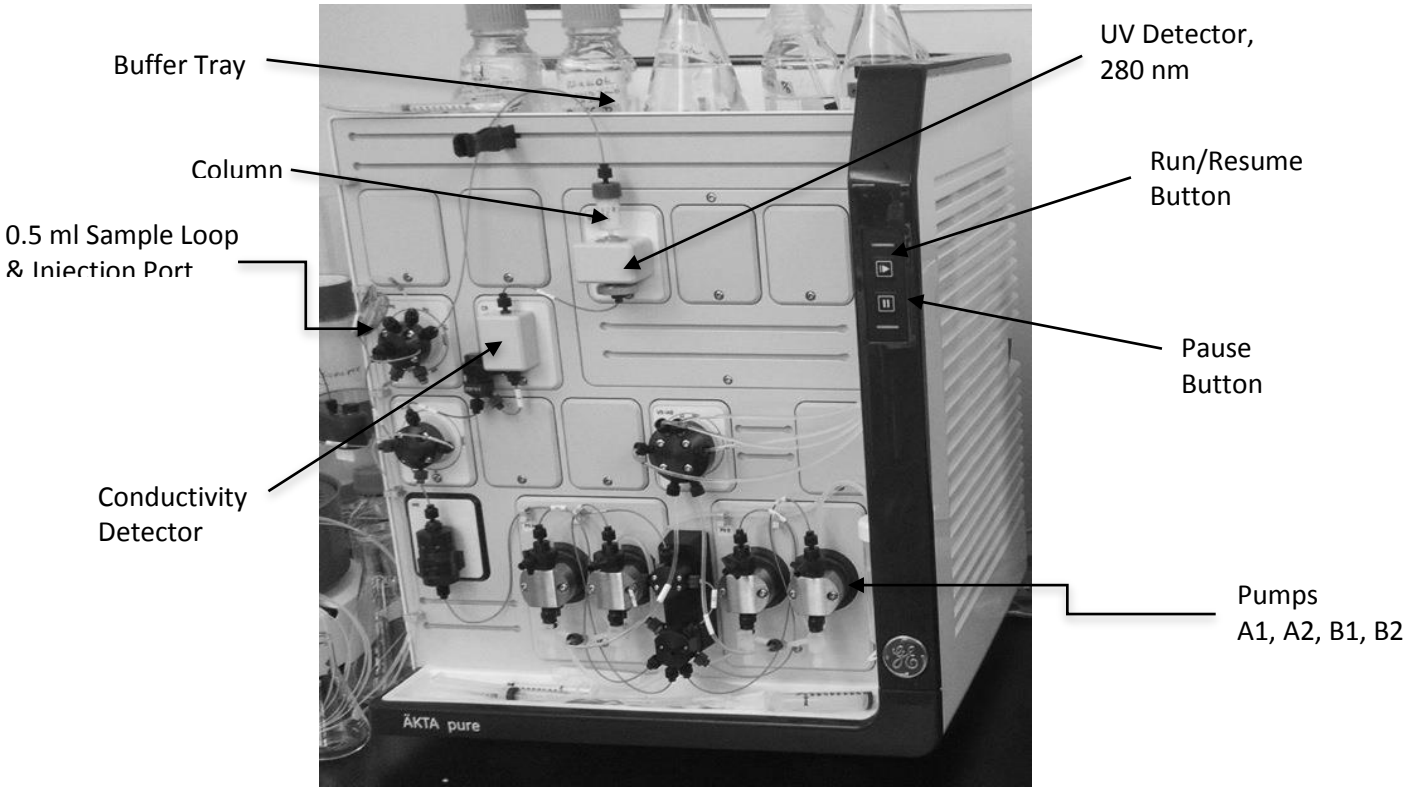
Attachments/Figures

Fig. 1. ***Diagram of AKTA pure instrument, fraction collector and computer***



SOP: Operation of ÄKTA pure Chromatography System for Cation Exchange Chromatography of t-PA

Fig. 2. ***AKTA pure Instrument Features***



SOP: Operation of ÄKTA pure Chromatography System for Cation Exchange Chromatography of t-PA

Fig 3. System Control window within the Unicorn 6.3 software.

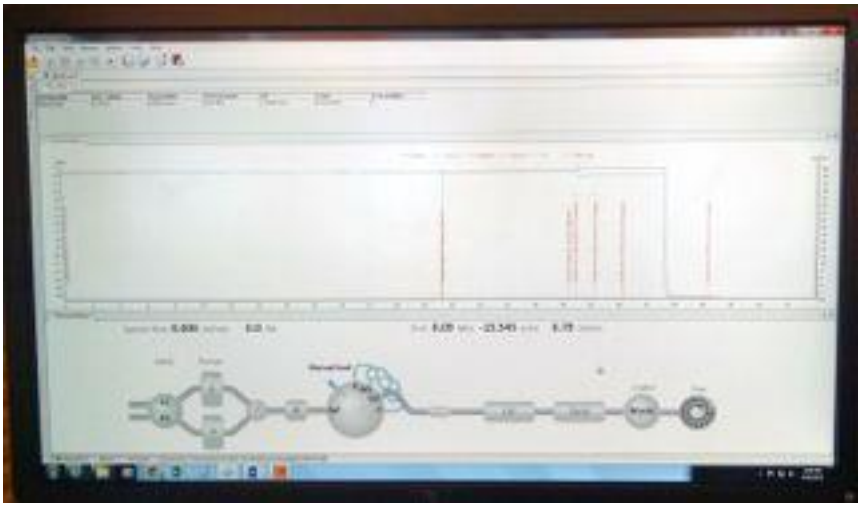
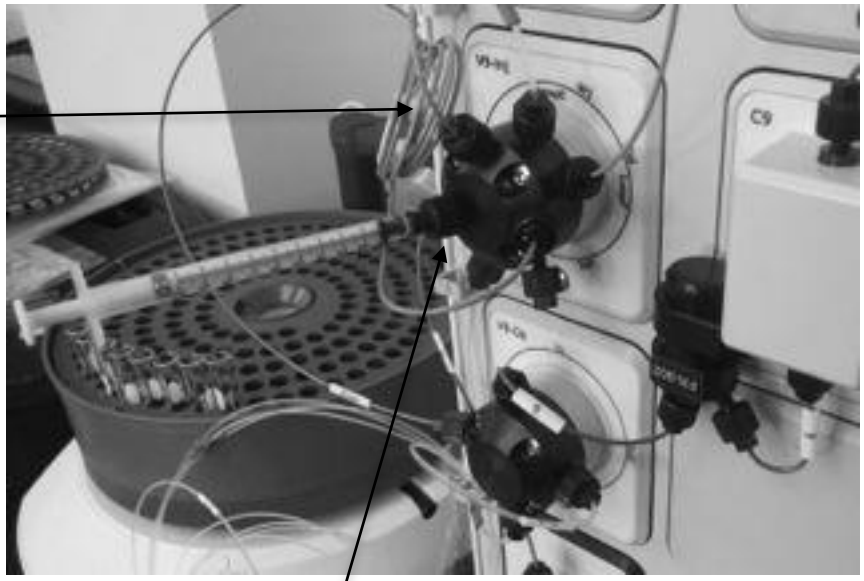


Fig. 4. ***Detail of Injection Port with Syringe in Place***.



SOP: Operation of ÄKTA pure Chromatography System for Cation Exchange Chromatography of t-PA

Fig. 5. ***Fraction collector carousel rubber advancement roller/gear.***

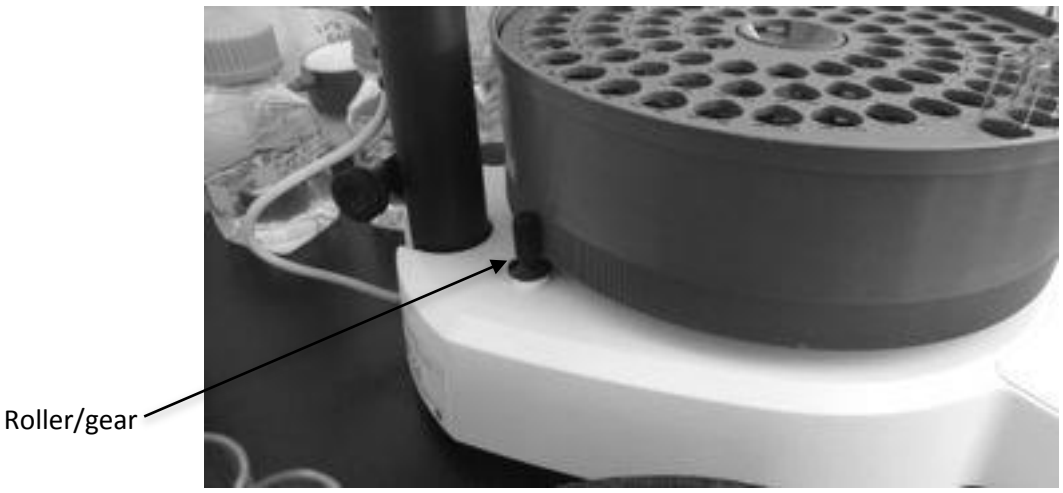
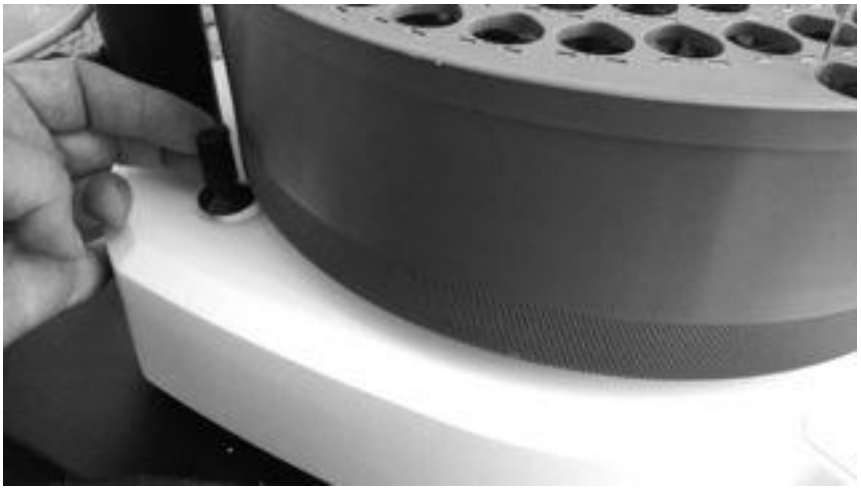


Fig. 6. Release of roller to allow free rotation of the carousel.



SOP: Operation of ÄKTA pure Chromatography System for Cation Exchange Chromatography of t-PA

Fig. 7. ***Location of tube #1 under the fraction collector drip outlet.***

10. History

Revision Number	*Effective Date*	*Preparer*	*Description of Change*
0	16JUL15	Dr. David Frank	Initial release

QC BIOCHEMISTRY

SOP: Human tPA Total Antigen ELISA Kit

Approvals

Preparer: Jason McMillan Date: 09APR14
Reviewer: Dr. Margaret Bryans Date: 10APR14

1. **Purpose**
   1.1. Quantitative determination of total tPA
2. **Scope and Applicability**
   2.1. Human tPA Total Antigen ELISA may be used for quantitative determination of total tPA in cell culture and tissue lysate samples as well as human plasma and other biological fluids.
3. **Summary of Method**
   3.1. Preparation of standard
   3.2. Standard and unknown addition
   3.3. Primary antibody addition
   3.4. Secondary antibody addition
   3.5. Substrate incubation
   3.6. Measurement
   3.7. Calculation of results
4. **References**
   4.1. Molecular Innovations Human tPA Total Antigen ELISA Kit (Cat # HTPAKT-TOT) Manual
   4.2. Bio Rad iMark Microplate Absorbance Reader SOP
5. **Precautions**
   5.1. None
6. **Responsibilities**
   6.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   6.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
7. **Equipment and Materials**
   7.1. Molecular Innovations Human tPA Total Antigen ELISA Kit (Cat # HTPAKT-TOT)
   7.2. 20µl, 200µl, and 1000µl pipettes and tips
   7.3. Shaking platform capable of reaching 300rpm
   7.4. Bio Rad iMark Microplate Absorbance Reader
   7.5. Microtubes and rack
   7.6. Blocking Buffer (3% BSA (w/v) in TBS buffer (0.1M Tris, 0.15M NaCl, pH 7.4))
   7.7. 1M HCl
   7.8. 1X washing Buffer
   7.9. tPA Samples from Spinner Flask and Bioreactor
8. **Procedure**
   8.1. Preparation of Standard (Dilutions for the standard curve and zero standard must be made and applied to the plate immediately)

SOP: Human tPA Total Antigen ELISA Kit

8.1.1. Combine 50µl of 1,000ng/ml standard with 150µl blocking buffer to create a 250ng/ml intermediate. Follow dilution table located in Attachments Section for standard preparation.

8.2. Remove microtiter plate from the bag and add 100µl of tPA standards and unknowns to their individual wells. Be sure to carefully record their position on the microtiter plate.

8.2.1. Shake plate at 300rpm for 30 minutes.

8.2.2. Wash wells three times with 300µl of wash buffer. Remove excess wash buffer by gently tapping microtiter plate on a paper towel or kimwipe.

8.3. Primary Antibody Addition

8.3.1. Add 100µl of primary antibody to all wells.

8.3.2. Shake plate at 300rpm for 30 minutes.

8.3.3. Wash wells three times with 300µl of wash buffer. Remove excess wash buffer by gently tapping microtiter plate on a paper towel or kimwipe.

8.4. Secondary Antibody Addition

8.4.1. Add 100µl of primary antibody to all wells.

8.4.2. Shake plate at 300rpm for 30 minutes.

8.4.3. Wash wells three times with 300µl of wash buffer. Remove excess wash buffer by gently tapping microtiter plate on a paper towel or kimwipe.

8.5. Substrate Incubation

8.5.1. Add 100µl of TMB substrate to all wells and shake at 300rpm for approximately 4 minutes. Substrate will change from colorless to different shades of blue.

8.5.2. Quench reaction by adding 50µl of 1M HCl in the same order as the substrate was added to stop the reaction when the sample shades of blue match the gradient of blue in the standards. The color will change from blue to yellow. Mix thoroughly by gently shaking the microtiter plate by hand for approximately one minute.

8.6. Measurement

8.6.1. Measure the absorbance in all wells at 450nm using the Bio Rad iMark Microplate Absorbance Reader.

8.7. Calculation of Results

8.7.1. Subtract the value of the zero point standard from all of the standards and unknowns to determine the corrected absorbance (A_{450}).

8.7.2. Plot A_{450} against the amount of tPA in the standards to create a Full Range Total Human tPA in BSA standard curve.

8.7.3. Fit a straight line through the linear points of the Full Range Total Human tPA in BSA standard curve to create a Linear Range Total Human tPA in BSA standard curve.

8.7.4. The amount of tPA in the unknowns can be determined from the standard curve.

8.7.5. Create a graph showing concentration of tPA over time in days.

9. Attachments

SOP: Human tPA Total Antigen ELISA Kit

tPA Concentration (ng/ml)	Dilutions
25	450µl (blocking buffer) + 50µl (250ng/ml)
10	300µl (blocking buffer) + 200µl (25ng/ml)
5	250µl (blocking buffer) + 250µl (10ng/ml)
2	300µl (blocking buffer) + 200µl (5ng/ml)
1	250µl (blocking buffer) + 250µl (2ng/ml)
0.5	250µl (blocking buffer) + 250µl (1ng/ml)
0.2	300µl (blocking buffer) + 200µl (0.5ng/ml)
0	250µl (blocking buffer) Zero point to determine background

Figure 1. Dilution table for preparation of human tPA standard

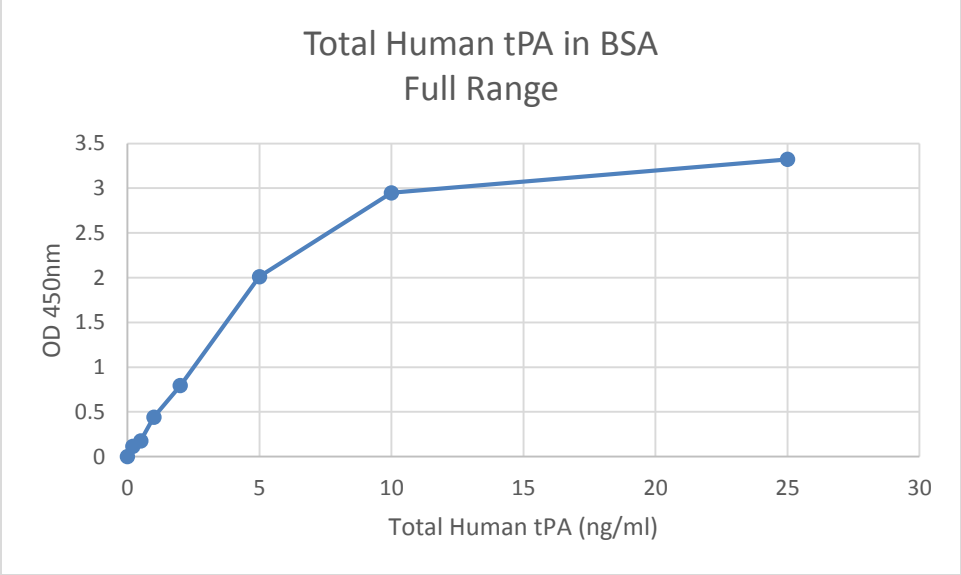


Figure 2. Total Human tPA in BSA Full Range (Example Only)

SOP: Human tPA Total Antigen ELISA Kit

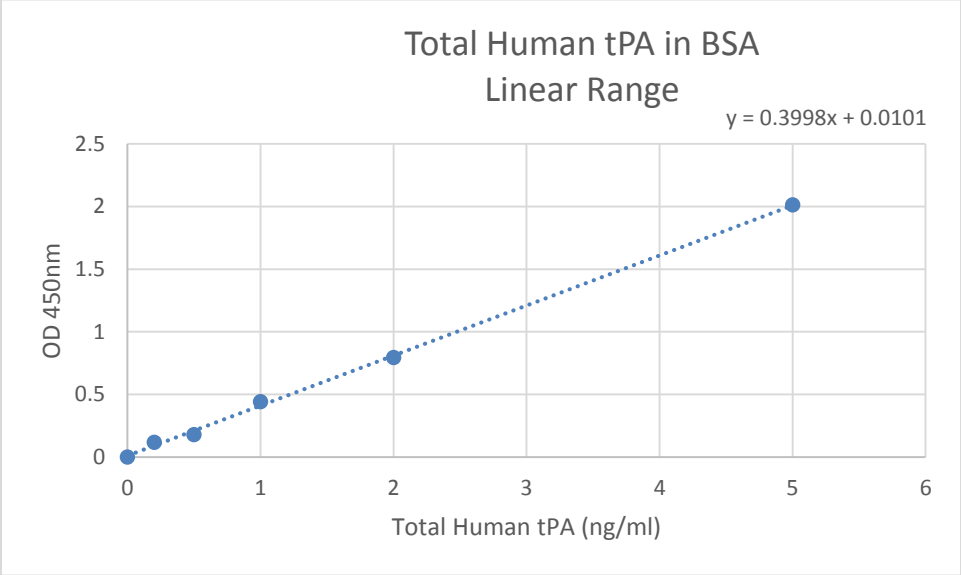


Figure 3. Total Human tPA in BSA Linear Range (Example Only)

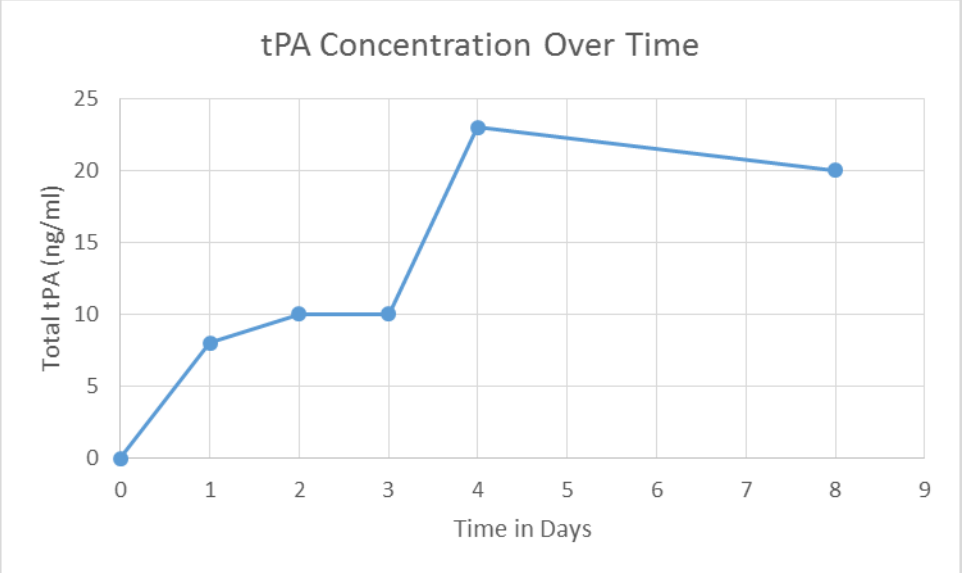


Figure 4. tPA Concentration Over Time (Example Only)

SOP: Human tPA Total Antigen ELISA Kit

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Figure 5. ELISA Plate Layout

10. History

Revision Number	*Effective Date*	*Preparer*	*Description of Change*
0	09APR14	Jason McMillan	Initial release

SOP: Human tPA Activity ELISA Kit

Approvals

Preparer: Jason McMillan Date: 10APR14
Reviewer: Dr. Margaret Bryans Date: 11APR14

1. **Purpose**
   1.1. Quantitative determination of active tPA
2. **Scope and Applicability**
   2.1. Human tPA Total Antigen ELISA may be used for quantitative determination of total tPA in cell culture and tissue lysate samples as well as human plasma and other biological fluids.
3. **Summary of Method**
   3.1. Biotinylated Human PAI-1 Addition
   3.2. Preparation of Standard
   3.3. Standard and unknown addition
   3.4. Primary antibody addition
   3.5. Secondary antibody addition
   3.6. Substrate incubation
   3.7. Measurement
   3.8. Calculation of results
4. **References**
   4.1. Molecular Innovations Human tPA Activity ELISA Kit (Cat # HTPAKT) Manual
   4.2. Bio Rad iMark Microplate Absorbance Reader SOP
5. **Precautions**
   5.1. None
6. **Responsibilities**
   6.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   6.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
7. **Equipment and Materials**
   7.1. Molecular Innovations Human tPA Activity ELISA Kit (Cat # HTPAKT)
   7.2. 20µl, 200µl, and 1000µl pipettes and tips
   7.3. Shaking platform capable of reaching 300rpm
   7.4. Bio Rad iMark Microplate Absorbance Reader
   7.5. Microtubes and rack
   7.6. Blocking Buffer (3% BSA (w/v) in TBS buffer (0.1M Tris, 0.15M NaCl, pH 7.4))
   7.7. 1M HCl
   7.8. 1X washing Buffer
   7.9. tPA Samples from Spinner Flask and Bioreactor

SOP: Human tPA Activity ELISA Kit

8. Procedure

8.1. Biotinylated Human PAI-1 Addition

8.1.1. Remove microtiter plate from the bag and add 100µl of Biotinylated Human PAI-1 to all wells.

8.1.2. Shake plate at 300rpm for 30 minutes.

8.1.3. Wash wells three times with 300µl of wash buffer. Remove excess wash buffer by gently tapping microtiter plate on a paper towel or kimwipe.

8.2. Preparation of Standard (Dilutions for the standard curve and zero standard must be made and applied to the plate immediately)

8.2.1. Dilute 100µl of 61 IU/ml standard with 900µl blocking buffer to create a 6.1 IU/ml standard. Follow dilution table located in Attachments Section for standard preparation.

8.3. Add 100µl of tPA standards and unknowns to their individual wells. Be sure to carefully record their position on the microtiter plate.

8.3.1. Shake plate at 300rpm for 30 minutes.

8.3.2. Wash wells three times with 300µl of wash buffer. Remove excess wash buffer by gently tapping microtiter plate on a paper towel or kimwipe.

8.4. Primary Antibody Addition

8.4.1. Add 100µl of primary antibody to all wells.

8.4.2. Shake plate at 300rpm for 30 minutes.

8.4.3. Wash wells three times with 300µl of wash buffer. Remove excess wash buffer by gently tapping microtiter plate on a paper towel or kimwipe.

8.5. Secondary Antibody Addition

8.5.1. Add 100µl of primary antibody to all wells.

8.5.2. Shake plate at 300rpm for 30 minutes.

8.5.3. Wash wells three times with 300µl of wash buffer. Remove excess wash buffer by gently tapping microtiter plate on a paper towel or kimwipe.

8.6. Substrate Incubation

8.6.1. Add 100µl of TMB substrate to all wells and shake at 300rpm for approximately 4 minutes. Substrate will change from colorless to different shades of blue.

8.6.2. Quench reaction by adding 50µl of 1M HCl in the same order as the substrate was added to stop the reaction when the sample shades of blue match the gradient of blue in the standards. The color will change from blue to yellow. Mix thoroughly by gently shaking the microtiter plate by hand for approximately one minute.

8.7. Measurement

8.7.1. Measure the absorbance in all wells at 450nm using the Bio Rad iMark Microplate Absorbance Reader.

8.8. Calculation of Results

8.8.1. Subtract the value of the zero point standard from all of the standards and unknowns to determine the corrected absorbance (A_{450}).

8.8.2. Plot A_{450} against the amount of active tPA in the standards to create a Full Range Active Human tPA in BSA standard curve.

SOP: Human tPA Activity ELISA Kit

8.8.3. Fit a straight line through the linear points of the Full Range Active Human tPA in BSA standard curve to create a Linear Range Active Human tPA in BSA standard curve.

8.8.4. The amount of tPA in the unknowns can be determined from the standard curve.

8.8.5. Create a graph showing activity of tPA over time in days.

9. Attachments

tPA Concentration (IU/ml)	µl of 6.1 IU/ml tPA Standard	µl of blocking buffer	Total volume (µl)
1	100	510	610
0.5	50	560	610
0.4	40	570	610
0.25	25	585	610
0.1	10	600	610
0.05	5	605	610
0.02	2	608	610
0.01	1	609	610
0	0	500	500

Figure 1. Dilution table for preparation of human tPA standard

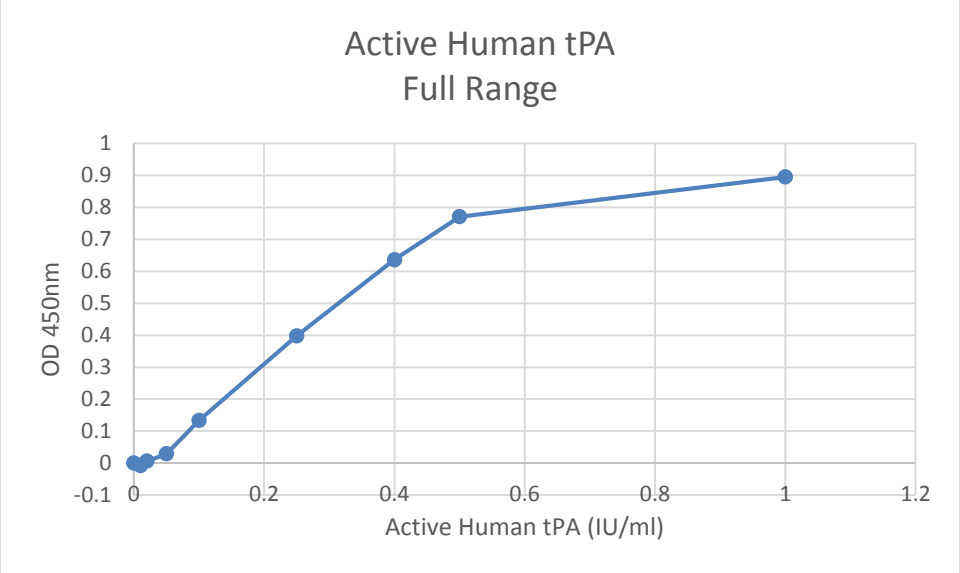


Figure 2. Active Human tPA Full Range (Example Only)

SOP: Human tPA Activity ELISA Kit

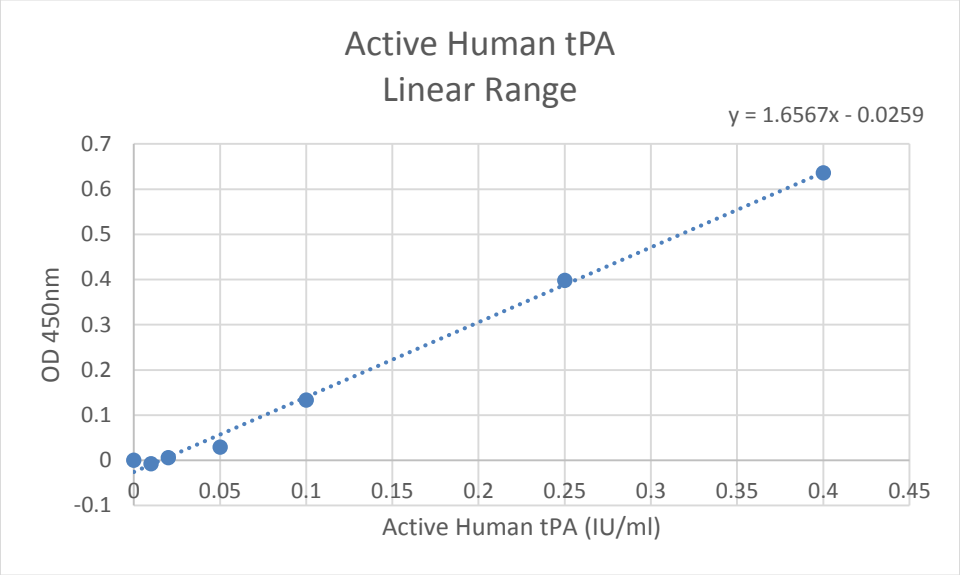


Figure 3. Active Human tPA Linear Range (Example Only)

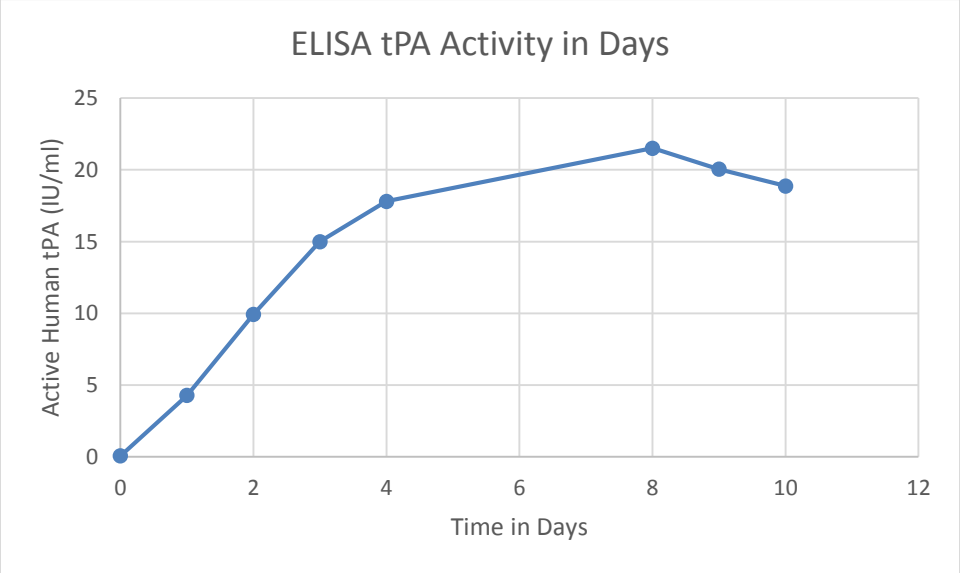


Figure 4. tPA Activity Over Time (Example Only)

SOP: Human tPA Activity ELISA Kit

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Figure 5. ELISA Plate Layout

10. History

Revision Number	*Effective Date*	*Preparer*	*Description of Change*
0	09APR14	Jason McMillan	Initial release

SOP: Glucose Determination Assay

Approvals:

Preparer: Jason McMillan Date: 26JUN14
Reviewer: Dr. Maggie Bryans Date: 27JUN14

1. **Purpose:**
   1.1. Use of the Glucose Determination Assay.
2. **Scope:**
   2.1. Applies to the quantitative determination of Glucose in serum.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. http://www.pointescientific.com/uploads/inserts/G7519-01-932.pdf
5. **Precautions:**
   5.1. The reagent should not be used if it has developed turbidity or other signs of microbial growth.
   5.2. The reagent should not be used if it fails to meet linearity claims or fails to recover control values in the stated range.
6. **Materials:**
   6.1. P-20 and P-1000 micropipette and tips
   6.2. Micro centrifuge tubes
   6.3. Timer
   6.4. Spectrophotometer able to read at 500nm
   6.5. Cuvettes
   6.6. Water bath (37°)
   6.7. Glucose reagent
   6.8. Glucose standard
   6.9. Control with known normal range
7. **Procedure:**
   7.1. **Running Assay**
      7.1.1. Turn on water bath and set to 37°C.
      7.1.2. Label micro centrifuge tubes “Blank,” Control,” Standard,” “Sample Name #’s.”
      7.1.3. Pipette 1.0ml of working reagent to all of the tubes and place in the 37°C water bath for 5 minutes.
      7.1.4. Remove micro centrifuge tubes from the water bath.
      7.1.5. Add 10µl of control solution to the “Control” tube, 10µl of Glucose standard to the “Standard” tube, and 10µl of sample to each of their respective “Sample” micro centrifuge tubes and mix by gently aspirating and dispensing the solution with the micropipette.
      7.1.6. Place all of the micro centrifuge tubes except for the “Blank” micro centrifuge tube back into the 37°C water bath for 10 minutes.

SOP: Glucose Determination Assay

7.1.7. Remove the micro centrifuge tubes from the 37°C water bath and immediately remove 1ml from each microfuge tube and place it in a corresponding labeled cuvette.

7.1.8. Read and record the absorbance of the tubes at 500nm using the "Blank" tube to zero the spectrophotometer.

7.1.9. Record absorbance values for each of the tubes and calculate the concentration of Glucose.

7.2. **Calculate Glucose Concentration.**

7.2.1. Formula to determine glucose concentration:

$$\text{Glucose (mg/dl)} = \frac{\text{Abs of sample}}{\text{Abs of standard}} \times \text{Concentration of standard (mg/dl)}$$

8. History:

Name	Date	Amendment
Jason McMillan	26JUN14	Initial release

Montgomery County Community College
340 DeKalb Pike
Blue Bell, PA 19422

SOP: Lactate Determination Assay

Approvals:

Preparer: Jason McMillan Date: 26JUN14
Reviewer: Dr. Maggie Bryans Date: 27JUN14

1. **Purpose:**
   1.1. Use of the Lactate Determination Assay.
2. **Scope:**
   2.1. Applies to the quantitative determination of Lactate in serum.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.4.1. http://www.pointescientific.com/uploads/inserts/L7596-01-984.pdf
5. **Precautions:**
   5.1. Reagents contain sodium azide as preservative. Upon disposal flush with large volumes of water.
   5.2. Do not use the reagents beyond the expiration date printed on the label.
6. **Materials:**
   6.1. P-20 and P-1000 micropipette and tips
   6.2. Micro centrifuge tubes
   6.3. Timer
   6.4. Spectrophotometer able to read at 550nm
   6.5. Cuvettes
   6.6. Heating block (37°)
   6.7. Lactate reagents R1 and R2
   6.8. Lactate standard
   6.9. Control with known normal range
7. **Procedure:**
   7.1. **Running Assay**
      7.1.1. Turn on heating block and set to 37°C.
      7.1.2. Label micro centrifuge tubes “Blank,” Control,” Standard,” “Sample Name #’s.”
      7.1.3. Prepare Lactate working reagent by combining R1 and R2 using a 3 to 2 ratio. Ex: Mix 3ml of R1 reagent with 2ml of R2 reagent.
      7.1.4. Pipette 1.0ml of working reagent to all of the tubes and place in the 37°C heating block for 5 minutes.
      7.1.5. Remove micro centrifuge tubes from the heating block.
      7.1.6. Add 10µl of control solution to the “Control” tube, 10µl of Lactate standard to the “Standard” tube, and 10µl of sample to each of their respective “Sample” micro centrifuge tubes and mix by gently aspirating and dispensing the solution with the micropipette.
      7.1.7. Place all of the micro centrifuge tubes except for the “Blank” micro centrifuge tube back into the 37°C heating block for 5 minutes.

SOP: Lactate Determination Assay

7.1.8. Remove the micro centrifuge tubes from the 37°C heating block and immediately remove 1ml from each microfuge tube and place it in a corresponding labeled cuvette.

7.1.9. Read and record the absorbance of the tubes at 550nm using the "Blank" tube to zero the spectrophotometer.

7.1.10. Record absorbance values for each of the tubes and calculate the concentration of Lactate.

7.2. **Calculate Lactate Concentration.**

7.2.1. Formula to determine lactate concentration:

$$\text{Lactate (mmol/L)} = \frac{\text{Abs of sample}}{\text{Abs of standard}} \times \text{Concentration of standard (mmol/L)}$$

7.2.2. If the result exceeds 20 mmol/L, the sample should be diluted 1:1 with normal saline, ran again, and the result multiplied by 2.

8. History:

Name	Date	Amendment
Jason McMillan	26JUN14	Initial release

SOP: Operation of Bio Rad iMark Microplate Absorbance Reader

Approvals

Preparer: Jason McMillan Date: 09APR14
Reviewer: Dr. Margaret Bryans Date: 09APR14

1. **Purpose**
   1.1. To measure absorbance values for use in determining Human tPA concentration and activity.
2. **Scope and Applicability**
   2.1. The Bio Rad iMark Microplate Absorbance Reader is an eight-channel, vertical path length photometer that measures the absorbance of the contents in the wells of a 96-well microtitration plates. It can perform single or dual wavelength measurements and can report absorbance values to three decimal places.
3. **Summary of Method**
   3.1. Turn on and load 96 well plate into Bio Rad iMark Microplate Absorbance Reader.
   3.2. Run protocol.
   3.3. Remove 96 well plate and turn off Bio Rad iMark Microplate Absorbance Reader
4. **References**
   4.1. iMark Microplate Absorbance Reader Instruction Manual
5. **Precautions**
   5.1. Be sure to open and close the "Reading Chamber Door" by pressing the "Open/Close" key on the "Keypad". Attempting to manually open or close the "Reading Chamber Door" can lead to damage.
6. **Responsibilities**
   6.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   6.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
7. **Equipment and Materials**
   7.1. iMark Microplate Absorbance Reader
   7.2. 96 Well Plate
8. **Procedure**
   8.1. Turn on the iMark Microplate Absorbance Reader by holding the "Power Button" until the "LCD" illuminates
      8.1.1. The "LCD" will display "Self Diagnosis" followed by "Initializing" Once initialization is complete, a login screen will appear.
      8.1.2. The login name will read "Common User" and a prompt for a password will be shown. Enter "00000" and press "Enter" on the "Keypad."
      8.1.3. "Lab Name, Kit Name, Reading Mode, and Measurement Filter" appears on screen (be sure the Measurement Filter is set at 450nm, the filter specific for tPA ELISAs), self-initialization is complete, and the Bio Rad iMark Microplate Absorbance Reader is ready for measurement.
   8.2. Open the "Reading Chamber Door" by pressing the "Open/Close" key on the "Keypad".

SOP: Operation of Bio Rad iMark Microplate Absorbance Reader

8.2.1. Once "Reading Chamber Door" is open, place 96 Well Plate into the "Reading Chamber" being sure to line the 96 Well Plate with the plate holding guides.

8.3. Close the "Reading Chamber Door" by pressing the "Open/Close" key on the "Keypad".

8.4. Start measurement reading by pressing the "Start/Stop" key on the "Keypad". The measurement function will begin and all data will be printed indicating the completion of the measurement function.

8.4.1. Remove data printout

8.5. Open the "Reading Chamber Door" by pressing the "Open/Close" key on the "Keypad".

8.5.1. Once "Reading Chamber Door" is open, remove 96 Well Plate from the "Reading Chamber."

8.6. Close the "Reading Chamber Door" by pressing the "Open/Close" key on the "Keypad".

8.7. Turn off the Bio Rad iMark Microplate Absorbance Reader by holding the "Power Button" until the "LCD" reads "Power Off" and "Yes" is highlighted, press "Enter" key. Bio Rad iMark Microplate Absorbance Reader will shut down.

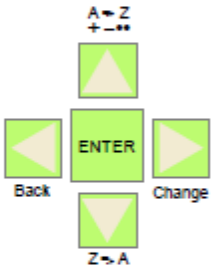
9. Attachments

Figure 1. External Features of Bio Rad iMark Microplate Absorbance Reader

SOP: Operation of Bio Rad iMark Microplate Absorbance Reader

Main	Returns to MAIN SCREEN.
Start/Stop	Initiates plate reading using current active protocol. Stops plate reading and printing.
Paper Feed	Advances paper strip in internal printer.
Print	Prints out the plate data and protocol information.
Edit	Enters the Edit Menu, and sets up the instrument.
Memory Recall	Reads out protocol and plate data.
Open/Close	Opens or closes the reading chamber door.

Up Arrow	Moves the cursor upward. Selects the alphabet or the symbol.
Left Arrow	Returns to the previous screen. Moves the cursor to the left.
Down Arrow	Moves the cursor downward. Selects the alphabet or the symbol.
Right Arrow	Moves the cursor to the right. Changes or selects the value and type.
Enter	Completes or seals a field entry.



Dot/Function	Inputs the dot. Changes the input mode.
Ten Keys	Inputs numbers or well type in plate mapping. 0 / EMP : Empty 5 / QC : QC control 1 / SMP : Sample 6 / CAL : Calibrator 2 / BLK : Blank 7 / CP : Positive Control 3 / STD : Standard 8 / CN : Negative Control 4 / CO : Cutoff control 9 / CW : Weak Positive Control

Figure 2. Keypad Guide for Bio Rad iMark Microplate Absorbance Reader

10. History

Revision Number	*Effective Date*	*Preparer*	*Description of Change*
0	09APR14	Jason McMillan	Initial release

SOP: Degassing a Solution by Helium Sparge

Approvals

Preparer: John Buford	Date: 24SEP13
Reviewer: Jack O'Neill	Date: 24SEP13
Reviewer: Tim Kull	Date: 25SEP13
Reviewer: Dr. Margaret Bryans	Date: 26SEP13

1. Purpose

1.1. Remove dissolved atmospheric gases from a solution by means of sparging with helium.

2. Scope and Applicability

2.1. Applicable to preparing solvent solutions for use in High Performance Liquid Chromatography (HPLC). Solution volume is limited to the range of 25 mL to 800 mL.

3. Summary of Method

3.1. Helium gas is delivered from a compressed gas tank to a porous metal sparger that is placed in a bottle of solution. Helium is bubbled through the solution for 15 minutes.

4. References

4.1. Airgas® Operation and Safety Instructions For Specialty Gas Regulation Equipment, form #320-517 Rev. 5/03

5. Definitions

Helium sparge	Using a stream of helium bubbles to sweep dissolved air out of liquids (helium is virtually insoluble in most HPLC solvent solutions, so very little helium replaces the air)
HPLC	High Performance Liquid Chromatography
PSI	Pounds per Square Inch

6. Precautions

6.1. A helium tank is compressed to pressures up to 2000 PSI. A sudden release of pressure can cause serious damage to personnel and equipment. Handle the helium tank and gas regulator with care and wear eye protection.

6.2. Helium sparging entails some risk of changing the composition of an HPLC solvent solution by selectively evaporating the more volatile components. Avoid an excessively vigorous flow of helium.

7. Responsibilities

7.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.

7.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

SOP: Degassing a Solution by Helium Sparge

Equipment and Materials

7.3. Laboratory grade (99.9+%) helium in a compressed gas cylinder (helium tank)
7.4. Airgas® gas cylinder regulator pre-connected to the helium tank
7.5. Porous metal sparger
7.6. Flexible gas tubing
7.7. Laboratory bottle containing HPLC storage solution (e.g. 50% MeOH/H_2O or 50% Acetonitrile/H_2O).
7.8. Solution to be sparged (25 to 800 mL)
7.9. Empty laboratory bottle (50, 100, 250, 500, or 1000 mL)
7.10. Two-hole stopper that is sized to the empty laboratory bottle (size # 1 for 50 or 100 mL bottle, size #6.5 for 250, 500, or 1000 mL bottle)
7.11. Laboratory grade water in a wash bottle
7.12. Waste beaker
7.13. Timer

8. Procedure

8.1. Verify that all personnel in the area are wearing eye protection.
8.2. Connect the sparger to the helium tank if it is not connected already:
(See Figure 1 for location of valves.)
8.2.1. Turn the gas pressure off by turning the regulator valve counter-clockwise for two full turns (in the direction marked "DECREASE").
8.2.2. Close the delivery valve by turning it clockwise until it stops.
8.2.3. Close the tank valve by turning it clockwise until it stops.
8.2.4. Connect one end of the flexible gas tubing to the gas regulator.
8.2.5. Connect the other end of the flexible gas tubing to the porous metal sparger.
8.2.6. Insert the gas tubing nearest the sparger through one of the stopper holes.
8.2.7. Place the sparger in the HPLC storage solution bottle and cap with the stopper.
8.3. Open the helium tank with the flow of helium turned off:
8.3.1. Verify that the gas pressure is turned off by turning the regulator valve counter-clockwise for one half turn (in the direction marked "DECREASE"). The valve knob should turn freely.
8.3.2. Close the delivery valve by turning it clockwise until it stops.
8.3.3. Slowly open the tank valve by turning it counter-clockwise.
8.4. Dispense the solution to a correctly sized laboratory bottle:
8.4.1. Select an empty laboratory bottle that has 20%-50% more volume than the quantity of solution to be sparged. (Note: additional volume is needed as head space to accommodate the sparger and bubbles without spilling.)
8.4.2. Measure the desired amount of solution and transfer it to the selected bottle. Verify that the bottle is at least 50% full and no more than 80% full.
8.5. Place the sparger in the solution bottle:
8.5.1. Remove the sparger from where it was stored in the HPLC storage solution bottle.
8.5.2. Rinse the sparger with water from a wash bottle over a waste beaker.
8.5.3. Place the sparger into the solution bottle and cap with the stopper.
8.5.4. Verify that the sparger is submerged in the solution and that the unused stopper hole is unobstructed.

SOP: Degassing a Solution by Helium Sparge

8.6. Turn on the flow of helium and set the deliver pressure to 3 PSI:
- 8.6.1. Verify that the gas pressure is turned off by turning the regulator valve counter-clockwise for one half turn (in the direction marked "DECREASE"). The valve knob should turn freely.
- 8.6.2. Slowly open the delivery valve by turning it counter-clockwise.
- 8.6.3. Watching the delivery pressure gauge, slowly increase the gas pressure by turning the regulator valve clockwise (in the direction marked "INCREASE") until the delivery gauge reads 3 PSI.
- 8.6.4. Observe bubbles rising from the sparger. Verify that there is sufficient head space in that bottle so that solution is not expelled from the bottle.

8.7. Sparge the solution for 15 minutes.

8.8. Turn off the flow of helium:
- 8.8.1. Turn the gas pressure off by turning the regulator valve counter-clockwise for two full turns (in the direction marked "DECREASE").
- 8.8.2. Close the delivery valve by turning it clockwise until it stops.

8.9. Store the sparger:
- 8.9.1. Remove the sparger from the solution bottle and cap the solution bottle.
- 8.9.2. Rinse the sparger with water from a wash bottle over a waste beaker.
- 8.9.3. Place the sparger in the HPLC storage solution bottle and cap with the stopper.

8.10. Close the tank valve by turning it clockwise until it stops.

SOP: Degassing a Solution by Helium Sparge

9. Attachments

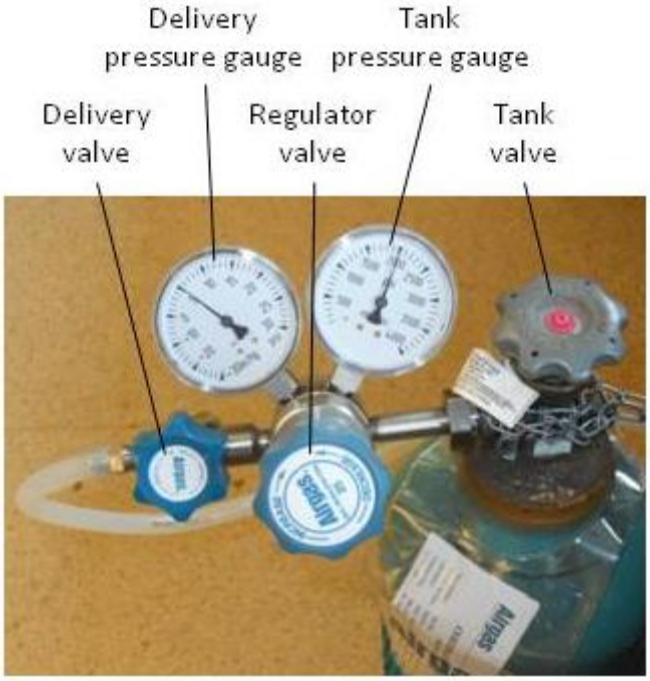


Figure 1. Helium Tank and Gas Regulator

Figure 2. Porous Metal Sparger

10. History

Revision Number	*Effective Date*	*Preparer*	*Description of Change*
0	09/25/2013	John Buford	Initial release

SOP: Buck Scientific BLC-20P HPLC Operation

Approvals

Preparer: John Buford	Date: 16OCT13
Reviewer: Tim Kull	Date: 30OCT13
Reviewer: Dr. Margaret Bryans	Date: 31OCT13

1. **Purpose**
   1.1. Basic operation of the Buck Scientific BLC-20P isocratic HPLC system in order to assay a sample using reverse phase high performance liquid chromatography (RP-HPLC).
2. **Scope and Applicability**
   2.1. High performance liquid chromatography (HPLC) is an analytical chemistry technique for separating the components of a liquid sample and for identifying and quantifying the components of the sample. This SOP provides the basic operations required to perform an assay using the Buck Scientific BLC-20P isocratic HPLC system, a reverse phase HPLC column, and a compatible mobile phase solution. Other process-specific SOPs are intended to provide the details of HPLC column selection, mobile phase solution preparation, sample preparation, flow rates, and run times.
3. **Summary of Method**
   3.1. Prepare the mobile phase and storage solutions
   3.2. Power up the HPLC system components and start the PeakSimple data collection software
   3.3. Equilibrate the system with mobile phase solution
   3.4. For each sample, run an assay:
      3.4.1. Use PeakSimple to start a new run
      3.4.2. Load and inject the sample
      3.4.3. Collecting data and store data
      3.4.4. Re-equilibrate the system if directed by the process-specific SOP
   3.5. Wash the system with storage solution
   3.6. Power down the system
4. **References**
   4.1. SOP: Degassing a Solution by Helium Sparge
   4.2. SUPPLEMENT to Operator's Manuals, BLC-20S PLUS Stack HPLC Systems, PN: DS090-0056, Rev A A333.

SOP: Buck Scientific BLC-20P HPLC Operation

5. Definitions

CV	Column Volume; the volume (mL) of the column containing the stationary phase; CV=2.91 mL for a standard size (4.6 X 250 mm) column
Equilibration	Running the mobile phase solution through the column prior to injecting the sample in order to bring the system into equilibrium
Flow rate	The rate (mL/min) that solution is pumped through the column. The operating flow rate is determined by the assay protocol, generally 1.0 mL/min for a standard size (4.6 X 250 mm) column
Helium sparge	Using a stream of helium bubbles to sweep dissolved air out of liquids (helium is virtually insoluble in most HPLC solvent solutions, so very little helium replaces the air)
HPLC	High Performance Liquid Chromatography
Isocratic	The composition of the mobile phase solution is constant; the system has only one pump.
Mobile phase	The solvent solution used to carry the sample through the column
PeakSimple	Software used to collect and display data
PSI	Pounds per Square Inch
Reverse phase chromatography	Separation based on hydrophobicity under conditions where the stationary phase is more hydrophobic than the mobile phase.
Stationary phase	The chromatography matrix through which the sample travels.

6. Precautions

6.1. Most solvents used for HPLC are toxic and flammable. For each solvent, read the Material Safety Data Sheet (MSDS) for hazards, handling and storage information. Wear personal protection equipment (PPE) and use a fume hood as required. Store solvents as indicated by the MSDSs.

6.2. HPLC systems operate at high pressures. Personnel injury and equipment damage can result if maximum pressure is exceeded or the pump runs dry. Monitor pressure readings and solution level whenever the pump is running. If pressure exceeds 2500 psi or if the solution runs out, stop the pump immediately by pressing the RUN/STOP button. Do not set the flow rate higher than 1.5 ml/min with a 250 mm column.

6.3. Different mobile phase solutions interact with the stationary phase differently, resulting in different back pressures for a given flow rate (see Table 3 for example pressure readings for various mobile phase solutions). When changing mobile phase solutions, monitor pressure readings carefully while running the first 5 CV of the new solution as it replaces the old solution in the lines and column.

6.4. Flow rate consistency is affected by the quality of the solutions. Use HPLC-grade solvents and filter solutions using a sub-micron filter (preferably 0.22 μm). Degas solutions prior to use.

6.5. To avoid microbial growth, do not leave the system in a high aqueous solution for a prolonged period. The system should be washed with a storage solution of 50% Methanol/H_20 or 50% Acetonitrile/H_20 if it is to be idle more than a few hours.

SOP: Buck Scientific BLC-20P HPLC Operation

7. Responsibilities

7.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.

7.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

8. Equipment and Materials

8.1. Buck Scientific BLC-20P HPLC system pre-configured with:

8.1.1. UV-Vis detector

8.1.2. Fluorometer (optional)

8.1.3. PeakSimple Chromatography Data System

8.1.4. Computer system with PeakSimple software installed

8.1.5. Reverse phase HLPC column

8.2. HPLC-grade solvent for mobile phase solution

8.3. HPLC-grade methanol for storage solution

8.4. HPLC-grade water

8.5. Chemically compatible sub-micron filters (preferably 0.22 μm)

8.6. 2 laboratory bottles for mobile phase solution and waste

8.7. 2 laboratory bottles for storage solution and waste

8.8. Small bottle for mobile phase (to be used for cleaning the sample syringe)

8.9. Sample overflow waste beaker

8.10. 5 mL Luer-Lok syringe

8.11. 100 μL HPLC sample syringe

8.12. Parafilm

8.13. Timer

9. Procedure

Note: this BLC-20P isocratic HPLC is configured with one pump, a UV-Vis detector, an add-on fluorescence detector, and a four channel serial port connected to a computer running PeakSimple software. The pump and UV-Vis detector are controlled by the HPLC front panel. Data is collected and displayed by the PeakSimple software on the computer.

9.1. ***Prepare mobile phase and storage solutions***:

9.1.1. A process-specific SOP should provide the composition and volume of mobile phase required. For an example, see Table 2. Example Solution Volume Calculations.

9.1.2. Prepare the mobile phase solution into a labeled laboratory bottle that is sized appropriately for degassing per the Degassing a Solution by Helium Sparge SOP. Filter the solution using a sub-micron filter (preferably 0.22 μm) that is chemically compatible.

9.1.3. Prepare a minimum of 300 mL 50% Methanol/H_20 or 50% Acetonitrile/H_20 storage solution into a labeled 500 mL laboratory bottle. Filter the solution using a sub-micron filter (preferably 0.22 μm) that is chemically compatible.

9.1.4. Degas both the mobile phase and storage solutions per the Degassing a Solution by Helium Sparge SOP.

SOP: Buck Scientific BLC-20P HPLC Operation

9.1.5. Transfer approximately 10 mL of mobile phase solution to a small labeled bottle to be used for rinsing the sample syringe.

9.1.6. Label an empty laboratory bottle as storage solution waste. Label another empty bottle as mobile phase solution waste.

9.2. ***Power up the HPLC system components and start the PeakSimple data collection software:***

9.2.1. Power up the computer system and monitor, then login.

9.2.2. Power up the pump unit using the switch located on the lower-right side in the back.

9.2.3. Power up the data system unit using the switch located on the upper-left side in the back.

9.2.4. Optionally, power up the fluorometer unit using the switch on the front.

9.2.5. Launch the PeakSimple data collection software:

9.2.5.1. Double click on the PeakSimple icon on the desktop. (Alternatively, navigate to C:\ Peak426-32bit and run Peak426-32bit.exe.) The PeakSimple window should appear as the software automatically connects to the HPLC hardware.

9.2.6. Allow the UV-Vis detector to warm up for 60 minutes prior to collecting data.

9.3. ***Switch the system to mobile phase solution:***

9.3.1. Verify that the pump is off. The Run LED should be off.

9.3.2. Place the intake line into the mobile phase solution bottle and cover with Parafilm. Verify that the frit is submerged in the solution.

9.3.3. Place the outlet line into the mobile phase waste bottle.

9.3.4. Place the sample overflow line into a small waste beaker.

9.4. ***Purge the intake line and prime the pump:***

9.4.1. Attach an empty 5 mL Luer-Lok syringe to the purge valve.

9.4.2. Open the prime/purge valve by turning it two full turns counter-clockwise.

9.4.3. Watching the intake line for bubbles, slowly draw the syringe plunger until it is fully drawn. Mobile phase solution and bubbles should fill the syringe.

9.4.4. Close the prime/purge value by rotating it clockwise until it stops.

9.4.5. Remove the syringe from the purge valve and expel the contents into the waste bottle.

9.4.6. Repeat attaching the syringe to the purge valve, drawing bubbles and solution into the syringe, and expelling into the waste bottle until free of bubbles (generally 10-to-15 mL of mobile phase are needed).

9.5. ***Start the pump and gradually increase the flow rate to the operating rate:***

9.5.1. A process-specific SOP should provide the flow rate for the mobile phase.

9.5.2. Set the initial flow rate to 0.1 mL/min:

9.5.2.1. Press the MODE button on the front panel repeatedly until the Flow LED turns on. The current flow rate (in ml/min) appears on the digital display.

9.5.2.2. Press the Flow up arrow button to increase the flow rate setting and press the down arrow button to decrease the flow rate setting.

9.5.2.3. Repeat pressing the Flow arrow buttons until 0.10 is displayed.

9.5.3. Start the pump by pressing the RUN/STOP button. The Run LED should turn on.

9.5.4. Monitor the pressure readings and solution level:

SOP: Buck Scientific BLC-20P HPLC Operation

9.5.4.1. Display the pressure reading by pressing the MODE button repeatedly until the Pressure LED turns on. The current pressure (in psi) appears on the digital display.
9.5.4.2. Verify that that solution is dripping into the waste bottle.
9.5.4.3. If pressure exceeds 2500 psi or if the solution runs out, stop the pump immediately by pressing the RUN/STOP button.

9.5.5. Gradually increase the flow rate in 0.1 mL/min increments over a period of 5 minutes to the specified flow rate:
9.5.5.1. Press the MODE button on the front panel repeatedly until the Flow LED turns on. The current flow rate (in ml/min) appears on the digital display.
9.5.5.2. Increase the flow rate setting by 0.1 mL/min by pressing the Flow up arrow button.
9.5.5.3. Monitor the pressure readings and solution level.
9.5.5.4. Repeat increasing the flow rate setting by 0.1 mL/min increments over a period of 5 minutes until the specified flow rate is achieved and pressure readings stabilize.

9.6. ***Set the UV-Vis detector wavelength and autozero the detector:***

9.6.1. A process-specific SOP should provide the assay run time and the UV-VIS detector wavelength.
9.6.2. Set the UV-Vis detector wavelength by pressing the λ up and down buttons on the front panel until the specified wavelength is displayed.
9.6.3. Autozero the UV-Vis detector by pressing the AUTOZERO button on the front panel.

9.7. ***Equilibrate the system by running mobile phase:***

9.7.1. A process-specific SOP should provide the equilibration run time. The run time may be expressed in terms of column volumes (CV); see Equation 1 for an example of converting a CV to a run time.
9.7.2. Operate the pump to run for the specified run time. Monitor the pressure readings and solution level. Monitor detector values and notify the instructor if the values appear to be unstable.
9.7.3. Alternatively, run an assay of a blank (see below) using mobile phase solution as the sample.

9.8. ***For each sample, run an assay:***

9.8.1. Use PeakSimple to start a new run and edit the run time:
9.8.1.1. Select File > New from the menu bar.
9.8.1.2. Select Edit > Channels… from the menu bar. The Channels dialog box should appear.
9.8.1.3. Select Channel 1: Details from the Channels dialog box. The Channel details dialog box should appear.
9.8.1.4. Enter the run time in the End time box.
9.8.1.5. Verify that the Remote start check box is checked.
9.8.1.6. Close the Channel details and Channels dialog boxes.

9.8.2. Autozero the UV-Vis detector by pressing the AUTOZERO button on the front panel.

SOP: Buck Scientific BLC-20P HPLC Operation

9.8.3. Load and inject the sample:
- 9.8.3.1. Verify that the injector port handle is set to the "Load" position.
- 9.8.3.2. Fill the HPLC sample syringe with 100 µL of sample, using care to avoid bubbles in the syringe.
- 9.8.3.3. Insert the syringe needle into the sample injection port. See Figure 4. Sample Injection Port with HLPC Syringe Attached.
- 9.8.3.4. Depress the syringe plunger, using care to avoid introducing bubbles. (Often there is a small bubble at the base of the plunger. Watch carefully and stop depressing the plunger before the bubble is loaded into the injector port. It is OK to leave a few µL of sample in the syringe.)
- 9.8.3.5. Turn the injector port handle clockwise from the "Load" to the "Inject" position. Note that the PeakSimple has started displaying the elapsed run time in upper right corner.
- 9.8.3.6. After 10 seconds, turn the injector port handle counter-clockwise from the "Inject" back to the "Load" position.
- 9.8.3.7. Remove the syringe from the sample injection port.
- 9.8.3.8. Rinse the syringe by filling it from the small bottle of mobile phase solution and expelling it into the mobile phase waste bottle at least three times.

9.8.4. Operate the pump for the specified run time. Monitor pressure readings and solution level. At the end of the run time, note that PeakSimple elapsed run time switches to STANDBY in upper right corner.

9.8.5. Use PeakSimple to view results and save the data to a chromatogram file:
- 9.8.5.1. Select View > Results… from the menu bar. The Results dialog box should appear.
- 9.8.5.2. Click the Copy button to copy the data.
- 9.8.5.3. Paste the data into an Excel spreadsheet.
- 9.8.5.4. Close the Results dialog box.
- 9.8.5.5. Select File > Save as… from the menu bar. The Save as dialog box should appear.
- 9.8.5.6. Enter a directory and a meaningful file name (e.g. operator initials, experiment name, and run number). Click the Save button.

9.8.6. Re-equilibrate the system if directed by the process-specific SOP.

9.8.7. Repeat this section for each sample.

9.9. ***Stop the pump:***

9.9.1. Press the RUN/STOP button. The Run LED should turn off and pressure readings should decrease gradually.

9.9.2. Monitor the pressure until it decreases to less than 100 psi.

9.10. ***Wash the system by running 5 CV of storage solution:***

9.10.1. Switch the system to storage solution per the instructions in section 9.2.6 above.

9.10.2. Purge the intake line and prime the pump per the instructions in section 9.4 above.

9.10.3. Set the initial flow rate to 0.1 mL/min.

9.10.4. Start the pump and gradually increase the flow rate to 0.5 mL/min over a period of 5 minutes.

9.10.5. Operate the pump for 30 minutes. Monitor pressure readings and solution level.

SOP: Buck Scientific BLC-20P HPLC Operation

9.10.6. Stop the pump and allow the pressure to decrease to less than 100 psi.

9.11. ***Power down the system:***

9.11.1. Exit PeakSimple by selecting File > Exit from the menu bar. A prompt should appear asking Save all before exiting? Click the No button.

9.11.2. Power down the pump unit using the switch located on the lower-right side in the back.

9.11.3. Power down the data system using the switch located on the upper-left side in the back.

Attachments

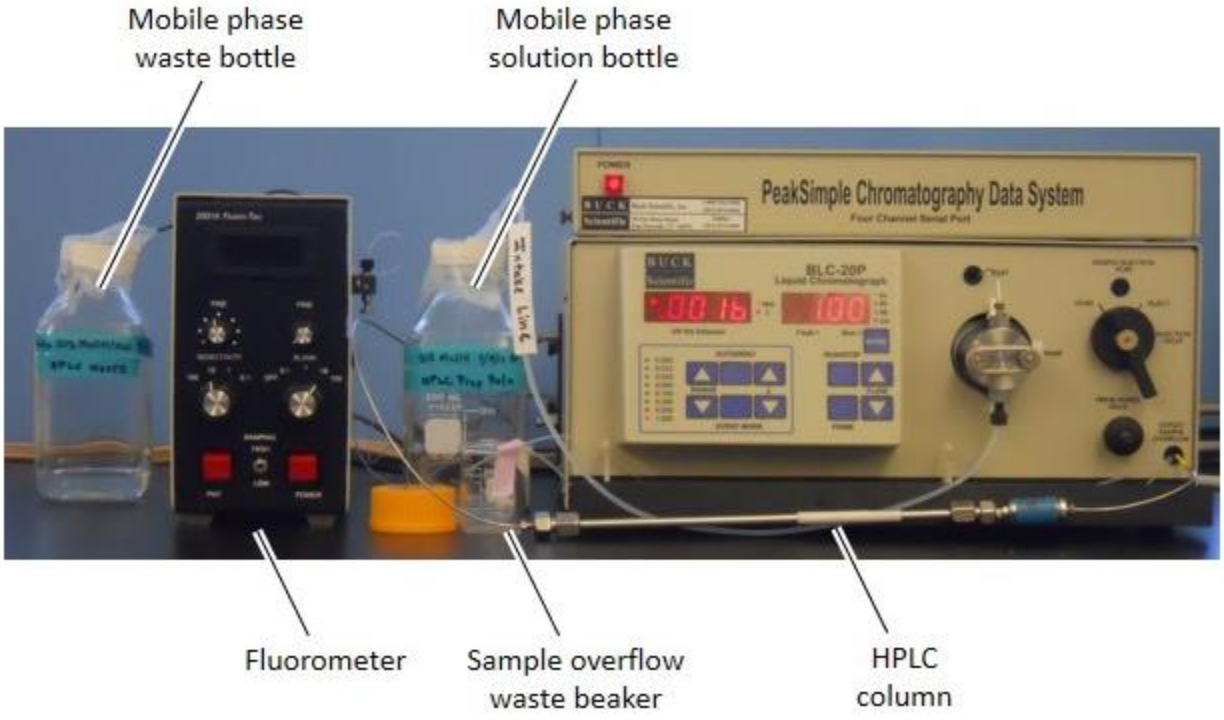


Figure 1. Buck Scientific BLC-20P HPLC System with Column and Fluorometer

SOP: Buck Scientific BLC-20P HPLC Operation

Figure 2. HPLC Front Panel

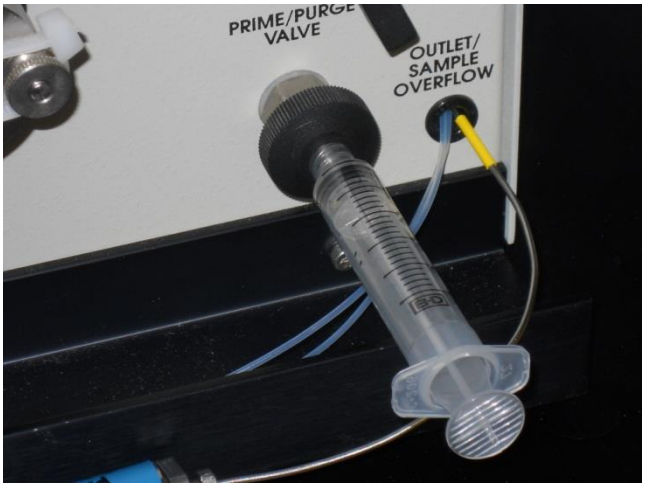


Figure 3. Purge Valve with Luer-Lok Syringe Attached

SOP: Buck Scientific BLC-20P HPLC Operation

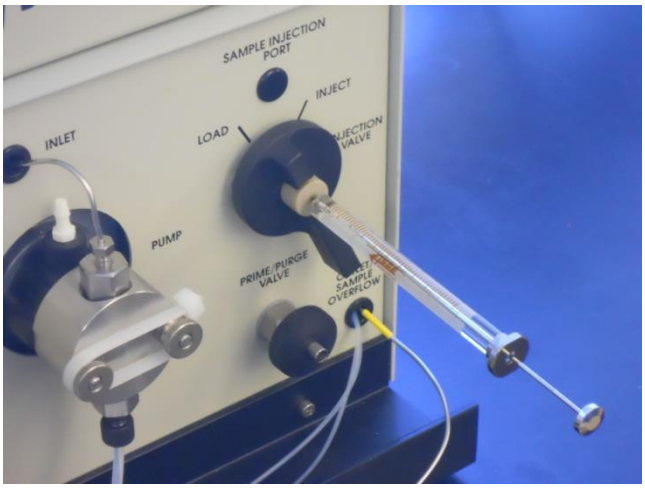


Figure 4. Sample Injection Port with HLPC Syringe Attached

Table 1. Common Column Volumes

Column dimension	Column volume (mL)
250 x 4.6 mm	2.91
150 x 4.6 mm	1.74
100 x 4.6 mm	1.16
50 x 4.6 mm	0.58
250 x 4.0 mm	2.20
125 x 4.0 mm	1.10
250 x 2.0 mm	0.55
150 x 2.0 mm	0.33
50 x 2.0 mm	0.11

$$CV = \pi r^2 L$$

where:

CV = column volume in mL
r = column radius in cm
L = column length in cm

SOP: Buck Scientific BLC-20P HPLC Operation

Table 2. Example Solution Volume Calculations

Mobile Phase solution		
Operation	***Calculation***	***Volume (ml)***
Prime and purge	20 mL	20
Increasing flow rate	0.75 mL/min * 5 min	4
Initial equilibration	15 CV = 15 * 3 mL	45
Assays	N * flow rate * run time = 4 * 0.75 mL/min * 15 min	45
Syringe cleaning	10 mL	10
Re-equilibration	N * 5 CV = 4 * 3 mL	12
Reserve volume	100 mL	100
Subtotal	Sum of the above	236
Minimum volume	Subtotal * 120%	**284**

Storage solution		
Operation	***Calculation***	***Volume (ml)***
Prime and purge	20 mL	20
Increasing flow rate	0.5 mL/min * 5 min	3
Wash	15 CV = 15 * 3 mL	45
Reserve volume	100 mL	100
Subtotal	Sum of the above	168
Minimum volume	Subtotal * 120%	**202**

Equation 1. Example Run Time Calculations

$$Run\ volume = 15\ CV$$
$$Column\ volume\ (CV) = 3\ mL$$
$$Flow\ rate = \ 0.75\ ml/min$$

$$Run\ time = \frac{Run\ volume}{Flow\ rate} = \frac{15\ CV\ *\ 3\ mL/CV}{0.75\ mL/min} = 60\ min.$$

Table 3. Example Flow Rates and Pressure Readings for a Haisil 100 C18 5µm 250 X 4.6mm Column

Mobile Phase Solution	***Flow Rate (mL/min)***	***Pressure Readings (psi)***
50% MeOH/H_2O	0.5	1400-1600
100% Isopropyl alcohol	0.5	1750-1950
100% H_2O	1.0	1600-1800
100% Acetonitrile	1.0	650-850
95% Hexane	1.0	500-700

SOP: Buck Scientific BLC-20P HPLC Operation

10. History

Revision Number	***Effective Date***	***Preparer***	***Description of Change***
0	31OCT13	John Buford	Initial release

SOP: Quantification of Caffeine in Coffee using HPLC

Approvals

Preparer: John Buford	Date: 23OCT13
Reviewer: Tim Kull	Date: 30OCT13
Reviewer: Dr. Margaret Bryans	Date: 31OCT13

1. Purpose

1.1. Quantify concentration of caffeine in a coffee sample using isocratic reverse phase high performance liquid chromatography (RP-HPLC) configured with a C18 column and a UV-Vis detector set for 275 nm.

2. Scope and Applicability

2.1. High performance liquid chromatography (HPLC) is an analytical chemistry technique for separating the components of a liquid sample and for identifying and quantifying the components of the sample. This SOP uses an HPLC to quantify the caffeine concentration in generic brewed coffee by assaying a series of caffeine standards in order to construct a calibration curve, and then assaying coffee samples and calculating caffeine concentration against the calibration curve. This SOP provides the details of HPLC column selection, mobile phase solution preparation, sample preparation, flow rates, and run times. Refer to the SOPs listed below for step-by-step HPLC operation instructions.

3. Summary of Method

3.1. Prepare mobile phase solution
3.2. Prepare caffeine standards and coffee samples
3.3. Power up the HPLC system and equilibrate with mobile phase solution
3.4. Run an assay for each of the caffeine standards and coffee samples
3.5. Wash the system with mobile phase solution
3.6. Graph the calibration curve
3.7. Compute the caffeine concentration of the coffee samples
3.8. Power down the system

4. References

4.1. SOP: Buck Scientific BLC-20P HPLC Operation, document QCB 7, revision 0, effective
4.2. SOP: Degassing a Solution by Helium Sparge, document number QCB 6, revision 0, effective 25SEP13.

5. Definitions

CV	Column Volume; the volume (mL) of the column containing the stationary phase; CV=2.91 mL for a standard size (4.6 X 250 mm) column
Equilibration	Running the mobile phase solution through the column prior to injecting the sample in order to bring the system into equilibrium
Flow rate	The rate (mL/min) that solution is pumped through the column. The operating flow rate is determined by the assay protocol.
Helium sparge	Using a stream of helium bubbles to sweep dissolved air out of liquids (helium is virtually insoluble in most HPLC solvent solutions, so very little helium replaces the air)
HPLC	High Performance Liquid Chromatography

SOP: Quantification of Caffeine in Coffee using HPLC

Isocratic	The composition of the mobile phase solution is constant; the system has only one pump.
Mobile phase	The solvent solution used to carry the sample through the column
PeakSimple	Software used to collect and display data
PSI	Pounds per Square Inch
Reverse phase chromatography	Separation based on hydrophobicity under conditions where the stationary phase is more hydrophobic than the mobile phase.
Stationary phase	The chromatography matrix through which the sample travels.

6. Precautions

6.1. HPLC systems operate at high pressures. Personnel injury and equipment damage can result if maximum pressure is exceeded or the pump runs dry. Monitor pressure readings and solution level whenever the pump is running. If pressure exceeds 2500 psi or if the solution runs out, stop the pump immediately by pressing the RUN/STOP button. Do not set the flow rate higher than 1.5 ml/min with a 250 mm column.

6.2. Flow rate consistency is affected by the quality of the solutions. Use HPLC-grade solvents and filter solutions using a sub-micron filter (preferably 0.22 μm). Degas solutions prior to use.

6.3. To avoid microbial growth, do not leave the system in a high aqueous solution for a prolonged period. The system should be washed with a storage solution of 50% Methanol/H_20 or 50% Acetonitrile/H_20 if it is to be idle more than a few hours.

6.4. Methanol is flammable. Can cause blindness if swallowed. Vapor is harmful. Irritating to skin and eyes.

7. Responsibilities

7.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.

7.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

8. Equipment and Materials

8.1. Buck Scientific BLC-20P HPLC system pre-configured with:

8.1.1. UV-Vis detector
8.1.2. PeakSimple Chromatography Data System
8.1.3. Computer system with PeakSimple software installed
8.1.4. Haisil 100 C18 5μm 250 X 4.6mm HPLC column

8.2. HPLC-grade methanol
8.3. HPLC-grade water
8.4. Laboratory-grade caffeine
8.5. 2 different samples of generic-brewed regular coffee
8.6. Sample overflow waste beaker
8.7. Analytic balance
8.8. 250 mL or 500 mL graduated cylinder
8.9. 500 mL volumetric flask
8.10. 100 mL volumetric flask

SOP: Quantification of Caffeine in Coffee using HPLC

8.11. 1 mL volumetric pipette
8.12. Stirring plate
8.13. 2 – 500 mL laboratory bottles (for mobile phase solution and waste)
8.14. Small laboratory bottle (for mobile phase to rinse the sample syringe)
8.15. 100 mL laboratory bottle (for stock caffeine solution)
8.16. Nalgene Rapid-flow filtration unit
8.17. 2 – 0.45 µm or 0.22 µm syringe filters
8.18. 2 – small beakers (for filtered coffee)
8.19. 6 – microfuge tubes (for caffeine standards and diluted coffee samples)
8.20. 5 mL Luer-Lok syringe
8.21. 100 µL HPLC sample syringe
8.22. Parafilm
8.23. Timer

9. Procedure

9.1. Prepare 500 mL 50% methanol/H_2O mobile phase solution (also to be used as storage solution):

9.1.1. Measure 250 mL HPLC-grade methanol using a graduated cylinder into a 500 mL volumetric flask.

9.1.2. Bring to volume 500 mL with HPLC-grade H_2O. Cover with parafilm and invert to mix. Check the volume and repeat. (When methanol and water combine, the total volume may be slightly less than the sum of the original volumes.)

9.1.3. Filter the mobile phase solution using a Nalgene Rapid-flow filtration unit. Transfer approximately 10 mL of mobile phase solution to a small labeled bottle to be used for rinsing the sample syringe. Transfer remaining solution to a labeled 500 mL laboratory bottle.

9.1.4. Label an empty bottle as mobile phase solution waste.

SOP: Quantification of Caffeine in Coffee using HPLC

9.2. Prepare 100 mL 1000 ppm caffeine stock solution:
- 9.2.1. Weigh 100 mg of caffeine on weighing paper or weighing boat using an analytic balance.
- 9.2.2. Transfer caffeine to a 100 mL volumetric flask.
- 9.2.3. Bring to volume 100 mL with mobile phase solution.
- 9.2.4. Rinse a stir bar, insert it into the flask, cover the flask with parafilm, and mix on a stir plate for 10 minutes. Remove the stir bar.
- 9.2.5. Filter the caffeine stock solution using the Nalgene Rapid-flow filtration unit. Transfer caffeine stock solution to a labeled 100 mL bottle.

9.3. Prepare 500 µL each of 200, 100, 50, and 25 ppm caffeine standards in labeled microfuge tubes as a serial dilution from 1000 ppm caffeine stock solution and mobile phase solution using a micropipette. Pipette up and down, then cap and vortex to mix each standard.

9.4. Filter approximately 5 mL of each coffee sample into a small labeled beaker using a 0.45 µm or 0.22 µm syringe filter, one beaker and filter per sample.

9.5. Prepare 1000 µL 20% coffee samples in labeled microfuge tubes by diluting the filtered coffee with mobile phase solution using a micropipette. Pipette up and down, then cap and vortex to mix each sample.

9.6. Degas the mobile phase solution per the Degassing a Solution SOP.

9.7. Power up the HPLC system and equilibrate with mobile phase solution for 30 minutes at the flow rate 0.5 mL/min:
- 9.7.1. Power up the HPLC system components and start the PeakSimple data collection software per the HPLC Operation SOP.
- 9.7.2. Switch the system to mobile phase solution per the HPLC Operation SOP.
- 9.7.3. Purge the intake line and prime the pump per the HPLC Operation SOP.
- 9.7.4. Start the pump and gradually increase the flow rate to 0.5 mL/min over 5 minutes per the HPLC Operation SOP.
- 9.7.5. Set the UV-Vis detector wavelength to 275 nm and autozero the detector per the HPLC Operation SOP.
- 9.7.6. Equilibrate the system with mobile phase solution at the flow rate 0.5 mL/min for 30 minutes per the HPLC Operation SOP. Monitor the UV-Vis detector readings.
- 9.7.7. Ensure that the UV-Vis detector warms up for 60 minutes prior to collecting data per the HPLC Operation SOP.

9.8. For each of the 25, 50, 100 and 200 ppm caffeine standards, run an assay for 10 minutes at 0.5 mL/min per the HPLC Operation SOP:
- 9.8.1. Use PeakSimple to start a new 10 minute run.
- 9.8.2. Autozero the UV-Vis detector.
- 9.8.3. Load and inject a caffeine standard.
- 9.8.4. Operate the pump for 10 minutes at 0.5 mL/min.
- 9.8.5. Note the time at the center of the caffeine peak on the chromatograph.
- 9.8.6. Save the data to a separate chromatogram file.
- 9.8.7. View the results and copy the data to a separate sheet in an Excel workbook.

SOP: Quantification of Caffeine in Coffee using HPLC

9.9. For each of the coffee samples, run an assay for 10 minutes each at 0.5 mL/min per the HPLC Operation SOP:
- 9.9.1. Use PeakSimple to start a new 10 minute run.
- 9.9.2. Autozero the UV-Vis detector.
- 9.9.3. Load and inject a coffee sample.
- 9.9.4. Operate the pump for 10 minutes at 0.5 mL/min.
- 9.9.5. Identify the caffeine peak on the chromatograph at the time noted above.
- 9.9.6. Save the data to a separate chromatogram file.
- 9.9.7. View the results and copy the data to a separate sheet in an Excel workbook.

9.10. Wash the system with mobile phase solution for 30 minutes at the flow rate 0.5 mL/min.

9.11. Graph the calibration curve using Excel:
- 9.11.1. Add a new sheet to the Excel workbook with the columns “Caffeine Standard” and “Area”.
- 9.11.2. Fill in the Caffeine Standard column with the values 25, 50, 100, and 200.
- 9.11.3. Fill in the Area column with the peak area values from the corresponding caffeine standard data collected above.
- 9.11.4. Use a scatter graph to display Caffeine Standard values on the x-axis and Area values on the y-axis.
- 9.11.5. Add a linear trendline to the graph, displaying the equation and R-squared value on the graph.

9.12. Compute the caffeine concentration of the coffee samples using the Excel TREND function.

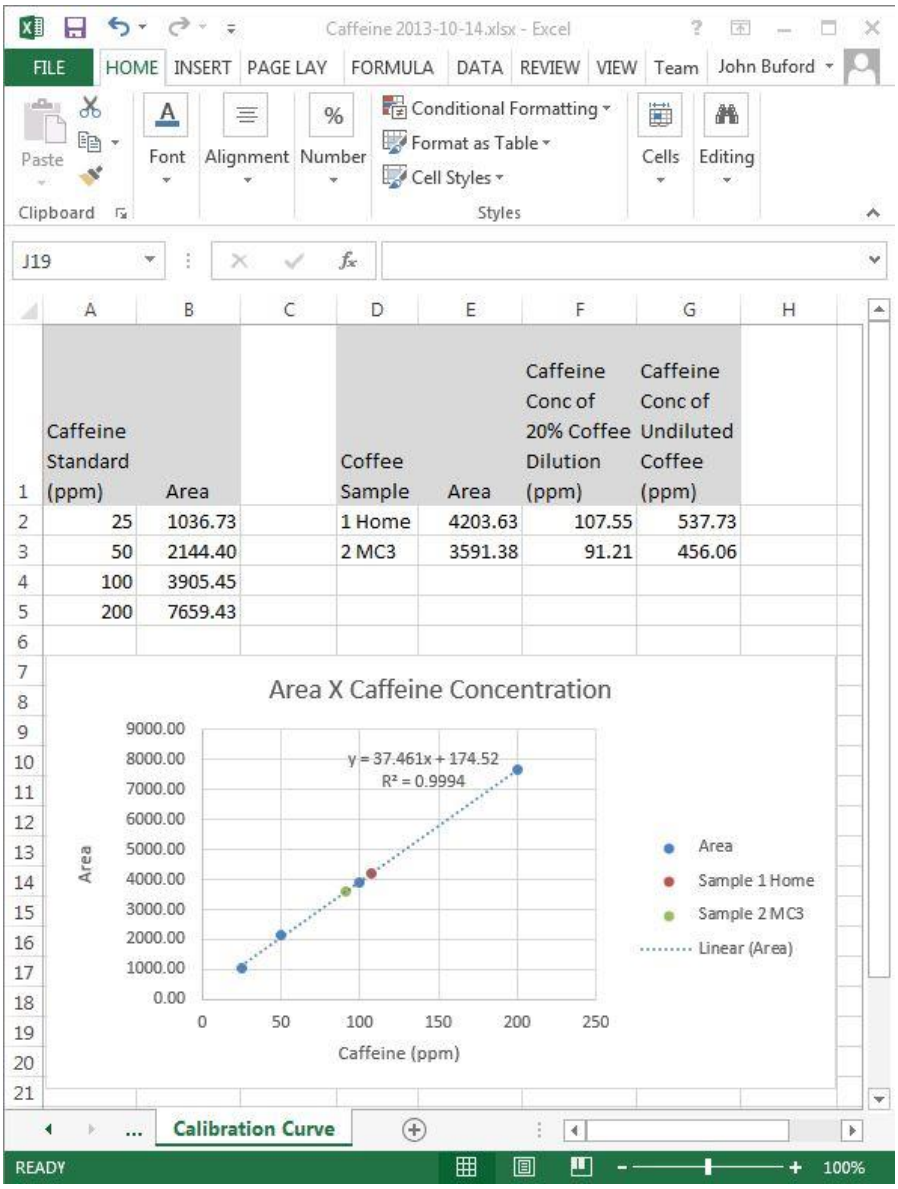
9.13. Stop the pump and allow the pressure to decrease to 0 per the HPLC Operation SOP.

9.14. Power down the system per the HPLC Operation SOP.

SOP: Quantification of Caffeine in Coffee using HPLC

10. Attachments

Figure 1. Example Calibration Curve



11. History

Revision Number	*Effective Date*	*Preparer*	*Description of Change*
0	31OCT13	John Buford	Initial release

SOP: Buck Scientific BLC-30G HPLC Operation

Approvals

Preparer: John Buford, Jason McMillan, Jack O'Neill Date: 16JUL15
Reviewer: Dr. Maggie Bryans Date: 17JUL15

1. **Purpose**
   1.1. Basic operation of the Buck Scientific BLC-30G gradient HPLC system in order to assay a sample using reverse phase high performance liquid chromatography (RP-HPLC).
2. **Scope and Applicability**
   2.1. High performance liquid chromatography (HPLC) is an analytical chemistry technique for separating the components of a liquid sample and for identifying and quantifying the components of the sample. This SOP provides the basic operations required to perform an assay using the Buck Scientific BLC-30G gradient HPLC system, a reverse phase HPLC column, and a compatible mobile phase solution. Other process-specific SOPs are intended to provide the details of HPLC column selection, mobile phase solution preparation, sample preparation, flow rates, and run times.
3. **Summary of Method**
   3.1. Prepare the mobile phase and storage solutions
   3.2. Power up the HPLC system components and start the PeakSimple data collection software
   3.3. Equilibrate the system with mobile phase solution
   3.4. For each sample, run an assay:
      3.4.1. Use PeakSimple to start a new run
      3.4.2. Load and inject the sample
      3.4.3. Collecting data and store data
      3.4.4. Re-equilibrate the system if directed by the process-specific SOP
   3.5. Wash the system with storage solution
   3.6. Power down the system
4. **References**
   4.1. SOP: Degassing a Solution by Helium Sparge
   4.2. BLC-30G Easy Installation and Quick Start Guide
5. **Definitions**

CV	Column Volume; the volume (mL) of the column containing the stationary phase; CV=2.91 mL for a standard size (4.6 X 250 mm) column
Equilibration	Running the mobile phase solution through the column prior to injecting the sample in order to bring the system into equilibrium
Flow rate	The rate (mL/min) that solution is pumped through the column. The operating flow rate is determined by the assay protocol, generally 1.0 mL/min for a standard size (4.6 X 250 mm) column
Helium sparge	Using a stream of helium bubbles to sweep dissolved air out of liquids (helium is virtually insoluble in most HPLC solvent solutions, so very little helium replaces the air)
HPLC	High Performance Liquid Chromatography

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Isocratic	The composition of the mobile phase solution is constant; the system has only one pump.
Mobile phase	The solvent solution used to carry the sample through the column
PeakSimple	Software used to collect and display data
PSI	Pounds per Square Inch
Reverse phase chromatography	Separation based on hydrophobicity under conditions where the stationary phase is more hydrophobic than the mobile phase.
Stationary phase	The chromatography matrix through which the sample travels.

6. Precautions

6.1. Most solvents used for HPLC are toxic and flammable. For each solvent, read the Material Safety Data Sheet (MSDS) for hazards, handling and storage information. Wear personal protection equipment (PPE) and use a fume hood as required. Store solvents as indicated by the MSDSs.

6.2. HPLC systems operate at high pressures. Personnel injury and equipment damage can result if maximum pressure is exceeded or the pump runs dry. Monitor pressure readings and solution level whenever the pump is running. If pressure exceeds 2500 psi or if the solution runs out, stop the pump immediately by pressing the RUN/STOP button. Do not set the flow rate higher than 1.5 ml/min with a 250 mm column.

6.3. Different mobile phase solutions interact with the stationary phase differently, resulting in different back pressures for a given flow rate (see Table 3 for example pressure readings for mobile phase solutions). When changing mobile phase solutions, monitor pressure readings carefully while running the first 5 CV of the new solution as it replaces the old solution in the lines and column.

6.4. Flow rate consistency is affected by the quality of the solutions. Use HPLC-grade solvents and filter solutions using a sub-micron filter (preferably 0.22 μm). Degas solutions prior to use.

6.5. To avoid microbial growth, do not leave the system in a high aqueous solution for a prolonged period. The system should be washed with a storage solution of 50% Methanol/H_20 or 50% Acetonitrile/H_20 if it is to be idle more than a few hours.

7. Responsibilities

7.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.

7.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

8. Equipment and Materials

8.1. Buck Scientific BLC-30G HPLC system pre-configured with:

8.1.1. UV-Vis detector
8.1.2. BLF-10 Fluorescence Detector
8.1.3. PeakSimple Chromatography Data System
8.1.4. Computer system with PeakSimple software installed
8.1.5. Reverse phase HLPC column

8.2. HPLC-grade solvent for mobile phase solution

SOP: Buck Scientific BLC-30G HPLC Operation

8.3. HPLC-grade methanol for storage solution
8.4. HPLC-grade water
8.5. Chemically compatible sub-micron filters (preferably 0.22 µm)
8.6. 2 laboratory bottles for mobile phase solution and waste
8.7. 2 laboratory bottles for storage solution and waste
8.8. Small bottle for mobile phase (to be used for cleaning the sample syringe)
8.9. Sample overflow waste beaker
8.10. 25 mL Luer-Lok syringe
8.11. 100 µL HPLC sample syringe
8.12. Parafilm
8.13. Timer

9. Procedure

Note: this BLC-30G Gradient HPLC is configured with two pumps, a UV-Vis detector, an add-on fluorescence detector, and a four channel serial port connected to a computer running PeakSimple software. The pumps and detectors are either controlled by the HPLC front panel (Figure 2) or the PeakSimple software, with the Run/Stop functions accessed through the Relay and Pump window. Data are collected and displayed by the PeakSimple software on the computer. For example, the UV-Vis data and Gradient functions are monitored/set through Channel 1. The FL data and Pump B functions are monitored/set through Channel 2. The Pressure is monitored through Channel 6.

9.1. ***Prepare mobile phase and storage solutions***:

9.1.1. A process-specific SOP should provide the composition and volume of mobile phase required. For an example, see Table 2. Example Solution Volume Calculations.

9.1.2. Prepare the mobile phase solution into a labeled laboratory bottle that is sized appropriately for degassing per the Degassing a Solution by Helium Sparge SOP. Filter the solution using a sub-micron filter (preferably 0.22 µm) that is chemically compatible.

9.1.3. Prepare a minimum of 300 mL 50% Methanol/H_20 solution into a labeled 500 mL laboratory bottle. Filter the solution using a sub-micron filter (preferably 0.22 µm).

9.1.4. Degas the mobile phase & storage solutions per the Degassing a Solution by Helium Sparge SOP.

9.1.5. Transfer approximately 10 mL of mobile phase solution to a small labeled bottle to be used for rinsing the sample syringe.

9.1.6. Label an empty laboratory bottle as storage solution waste. Label another empty bottle as mobile phase solution waste.

9.2. ***Power up the HPLC system components and start the PeakSimple data collection software:***

9.2.1. Power up the computer system and monitor, then login.

9.2.2. Power up the pump units UV-Vis detector, and fluorescence detector using the red switch located on the power strip to the left of the unit.

9.3. **Launch the PeakSimple data collection software:**

SOP: Buck Scientific BLC-30G HPLC Operation

9.3.1.1. Double click on the PeakSimple icon on the desktop. (Alternatively, navigate to C:\ Peak426-32bit and run Peak426-32bit.exe.) The PeakSimple window should appear as the software automatically connects to the HPLC hardware.
9.3.1.2. Navigate to "Edit" in the top left corner and select "Channels" from the dropdown menu.
9.3.1.3. Next to Channel 1:Uv-Vis, ensure that "active," "display" and "integrate" all have checks in them.
9.3.1.4. Select "Details"
9.3.1.5. Under the section "Control by" select "Gradient"
9.3.1.6. Under "End Time" input a value in minutes for the length of the run.
9.3.1.7. Press "OK" when finished.
9.3.1.8. Select "Gradient"
9.3.1.9. Select "Clear"
9.3.1.10. Select "Add"
9.3.1.11. Fill in the values to create the desired gradient.
9.3.1.12. If multiple gradient steps are necessary, repeat steps 9.2.3.9. and 9.2.3.10.
9.3.1.13. Press "OK" when finished.
9.3.1.14. Next to Channel 2: Fluorescence, ensure that "active," "display" and "integrate" all have checks in them.
9.3.1.15. Select "Details"
9.3.1.16. Under the section "Control by" select "Gradient"
9.3.1.17. Under "End Time" input a value in minutes for the length of the run.
9.3.1.18. Press "OK" when finished.
9.3.1.19. Next to Channel 6, ensure that "active," and "display" have checks in them.
9.3.1.20. Select "Details"
9.3.1.21. Ensure that "Datalogger mode" has a check in the box next to it.
9.3.1.22. Ensure Offset = 0
9.3.1.23. Ensure Gain = 1
9.3.1.24. Ensure Decimal Places = 0
9.3.1.25. Under "End Time" input a value in minutes for the length of the run.
9.3.1.26. Press "OK" when finished.
9.3.1.27. Press "OK" to close the Channels window.
9.3.1.28. Allow the detectors to warm up for 60 minutes prior to collecting data.

9.4. ***Switch the system to mobile phase solution:***

9.4.1. Verify that the pump is off. The Run LED should be off.
9.4.2. Place the intake lines into the mobile phase solution bottle and cover with Parafilm. Verify that the frit is submerged in the solution.
9.4.3. Place the outlet line into the mobile phase waste bottle.

SOP: Buck Scientific BLC-30G HPLC Operation

9.4.4. Place the sample overflow line into a small waste beaker.

9.5. ***Purge the intake line and prime the pump:***

9.5.1. Attach an empty 25 mL Luer-Lok syringe to the purge valve (Figure 3).
9.5.2. Open the prime/purge valve by turning it two full turns counter-clockwise.
9.5.3. Watching the intake line for bubbles, slowly draw the syringe plunger until it is fully drawn. Mobile phase solution and bubbles should fill the syringe.
9.5.4. Close the prime/purge value by rotating it clockwise until it stops. Stop the pumps.
9.5.5. Remove the syringe from the purge valve and expel the contents into the waste bottle.
9.5.6. Repeat attaching the syringe to the purge valve, drawing bubbles and solution into the syringe, and expelling into the waste bottle until free of bubbles (generally 20-to-30 mL of mobile phase are needed).

9.6. ***Start the pump and gradually increase the flow rate to the operating rate:***

9.6.1. A process-specific SOP should provide the flow rate for the mobile phase.
9.6.2. Set the initial flow rate to 0.1 mL/min:
9.6.2.1. Press the MODE button on the front panel repeatedly until the Flow LED turns on. The current flow rate (in ml/min) appears on the digital display.
9.6.2.2. Press the Flow up arrow button to increase the flow rate setting and press the down arrow button to decrease the flow rate setting.
9.6.2.3. Repeat pressing the Flow arrow buttons until 0.10 is displayed.
9.6.3. Start the pump by pressing the RUN/STOP button. The Run LED should turn on.
9.6.4. Monitor the pressure readings and solution level:
9.6.4.1. Display the pressure reading by pressing the MODE button repeatedly until the Pressure LED turns on. The current pressure (in psi) appears on the digital display.
9.6.4.2. Verify that that solution is dripping into the waste bottle.
9.6.4.3. If pressure exceeds 2500 psi or if the solution runs out, stop the pump immediately by pressing the RUN/STOP button.
9.6.5. Gradually increase the flow rate in 0.1 mL/min increments over a period of 5 minutes to the specified flow rate:
9.6.5.1. Press the MODE button on the front panel repeatedly until the Flow LED turns on. The current flow rate (in ml/min) appears on the digital display.
9.6.5.2. Increase the flow rate setting by 0.1 mL/min by pressing the Flow up arrow button.
9.6.5.3. Monitor the pressure readings and solution level.
9.6.5.4. Repeat increasing the flow rate setting by 0.1 mL/min increments over a period of 5 minutes until the specified flow rate is achieved and pressure readings stabilize.

9.7. ***Set the UV-Vis detector wavelength and autozero the detector:***

9.7.1. A process-specific SOP should provide the assay run time and the UV-VIS detector wavelength.
9.7.2. Set the UV-Vis detector wavelength by pressing the λ up and down buttons on the front panel until the specified wavelength is displayed.

SOP: Buck Scientific BLC-30G HPLC Operation

9.7.3. Autozero the UV-Vis detector by pressing the AUTOZERO button on the front panel.

9.8. ***Equilibrate the system by running mobile phase:***

9.8.1. A process-specific SOP should provide the equilibration run time. The run time may be expressed in terms of column volumes (CV); see Equation 1 for an example of converting a CV to a run time.

9.8.2. Operate the pump to run for the specified run time. Monitor the pressure readings and solution level. Monitor detector values and notify the instructor if the values appear to be unstable.

9.8.3. Alternatively, run an assay of a blank (see below) using mobile phase solution as the sample.

9.9. ***For each sample, run an assay:***

9.9.1. Use PeakSimple to start a new run and edit the run time:

9.9.1.1. Select File > New from the menu bar.

9.9.1.2. Select Edit > Channels… from the menu bar. The Channels dialog box should appear.

9.9.1.3. Select Channel 1: Details from the Channels dialog box. The Channel details dialog box should appear.

9.9.1.4. Ensure previously set values are still in place.

9.9.1.5. Verify that the "Remote start" check box is checked.

9.9.1.6. Press "OK" when finished.

9.9.1.7. Select Channel 2: Details from the Channels dialog box. The Channel details dialog box should appear.

9.9.1.8. Ensure previously set values are still in place.

9.9.1.9. Verify that the "Remote start" check box is checked.

9.9.1.10. Press "OK" when finished.

9.9.1.11. Select Channel 6: Details from the Channels dialog box. The Channel details dialog box should appear.

9.9.1.12. Ensure previously set values are still in place.

9.9.1.13. Verify that the "Remote start" check box is checked.

9.9.1.14. Press "OK" when finished.

9.9.1.15. Press "OK" to close the Channels window.

9.9.2. Autozero the UV-Vis detector by pressing the AUTOZERO button on the front panel.

9.9.3. Load and inject the sample:

9.9.3.1. Verify that the injector port handle is set to the "Load" position.

9.9.3.2. Fill the HPLC sample syringe with 100 µL of sample, using care to avoid bubbles in the syringe.

9.9.3.3. Insert the syringe needle into the sample injection port (Figure 4).

9.9.3.4. Depress the syringe plunger, using care to avoid introducing bubbles. (Often there is a small bubble at the base of the plunger. Watch carefully and stop depressing the plunger before the bubble is loaded into the injector port. It is OK to leave a few µL of sample in the syringe.)

SOP: Buck Scientific BLC-30G HPLC Operation

9.9.3.5. Turn the injector port handle clockwise from the "Load" to the "Inject" position (Figure 5). Note, the PeakSimple software has started displaying the elapsed run time in upper right corner.

9.9.3.6. After 10 seconds, turn the injector port handle counter-clockwise from the "Inject" back to the "Load" position.

9.9.3.7. Remove the syringe from the sample injection port.

9.9.3.8. Rinse the syringe by filling it from the small bottle of mobile phase solution and expelling it into the mobile phase waste bottle at least three times.

9.9.4. Operate the pump for the specified run time. Monitor pressure readings and solution level. At the end of the run time, note that PeakSimple elapsed run time switches to STANDBY in upper right corner.

9.9.5. Use PeakSimple to view results and save the data to a chromatogram file:

9.9.5.1. Select View > Results… from the menu bar. The Results dialog box should appear.

9.9.5.2. Click the Copy button to copy the data.

9.9.5.3. Paste the data into an Excel spreadsheet.

9.9.5.4. Close the Results dialog box.

9.9.5.5. Select File > Save as… from the menu bar. The Save as dialog box should appear.

9.9.5.6. Enter a directory and a meaningful file name (e.g. operator initials, experiment name, and run number). Click the Save button.

9.9.6. Re-equilibrate the system if directed by the process-specific SOP.

9.9.7. Repeat this section for each sample.

9.10. ***Stop the pump:***

9.10.1. Press the RUN/STOP button. The Run LED should turn off and pressure readings should decrease gradually.

9.10.2. Monitor the pressure until it decreases to less than 100 psi.

9.11. ***Wash the system by running 5 CV of storage solution:***

9.11.1. Switch the system to storage solution per the instructions in section 9.4 above.

9.11.2. Purge the intake line and prime the pump per the instructions in section 9.5 above.

9.11.3. Set the initial flow rate to 0.1 mL/min.

9.11.4. Start the pump and gradually increase the flow rate to 0.5 mL/min over a period of 5 minutes.

9.11.5. Operate the pump for 30 minutes. Monitor pressure readings and solution level.

9.11.6. Stop the pump and allow the pressure to decrease to less than 100 psi.

9.12. ***Power down the system:***

9.12.1. Exit PeakSimple by selecting File > Exit from the menu bar. A prompt should appear asking Save all before exiting? Click the No button.

9.12.2. Power down the pump units and the detectors using the red switch located on the power strip to the left of the pumps.

9.12.3. Power down the Computer system.

SOP: Buck Scientific BLC-30G HPLC Operation

10. Attachments

Figure 1. Buck Scientific BLC-20G HPLC System with Pumps and Detectors

SOP: Buck Scientific BLC-30G HPLC Operation

Figure 2. HPLC Front Panel

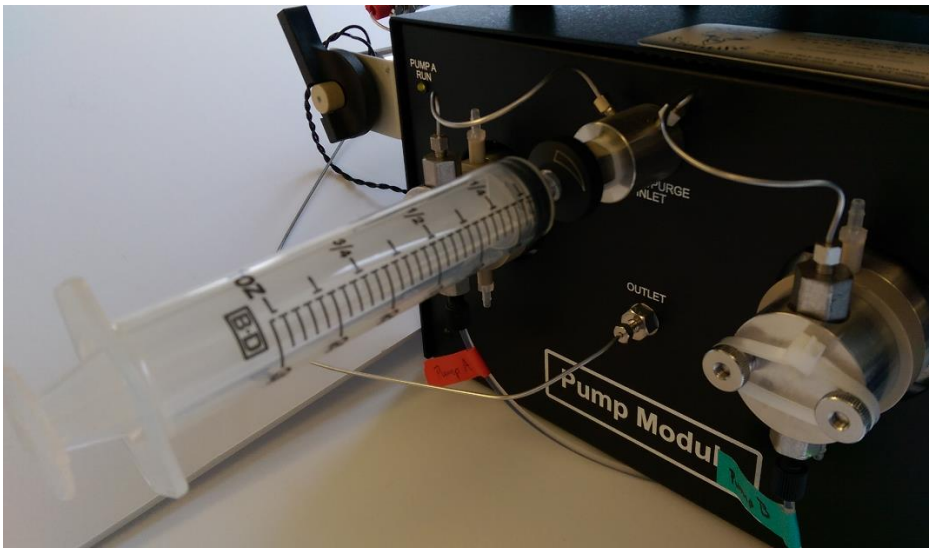


Figure 3. Purge Valve with 25 mL Luer-Lok Syringe Attached

SOP: Buck Scientific BLC-30G HPLC Operation

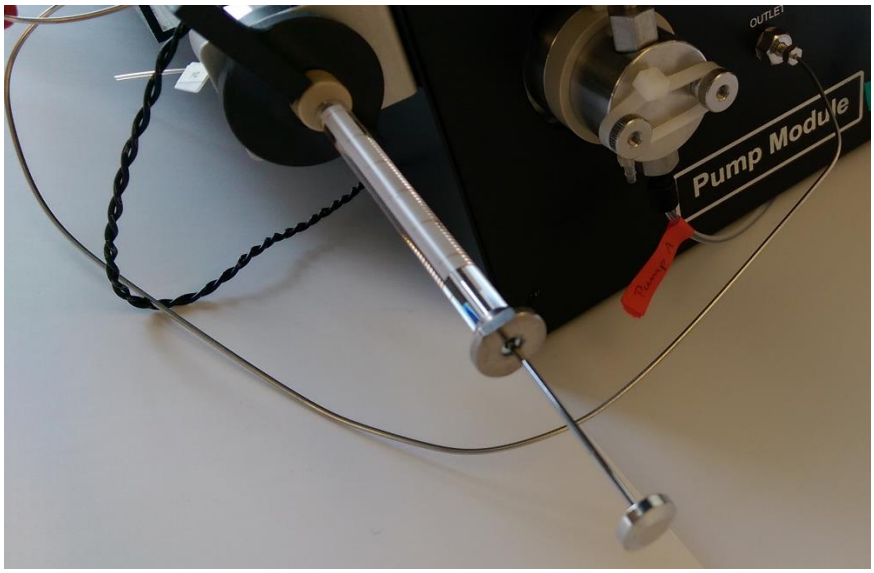
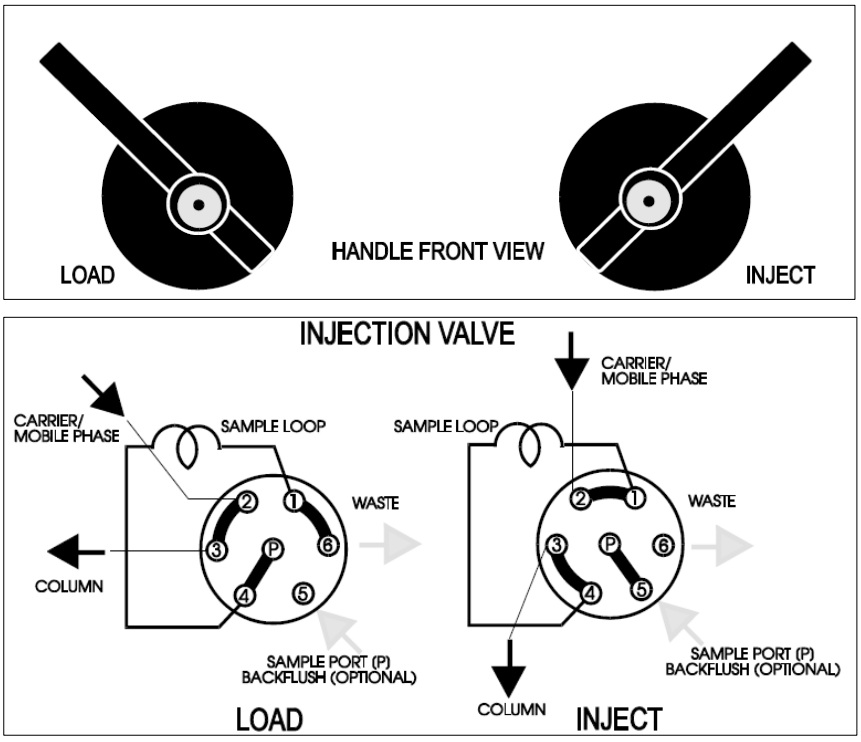


Figure 4. Sample Injection Port with HLPC Syringe Attached in 'Load' Position



Figures 5. Sample Injection Port Positions: 'Load' and 'Inject'

SOP: Buck Scientific BLC-30G HPLC Operation

Table 1. Common Column Volumes

Column dimension	Column volume (mL)
250 x 4.6 mm	2.91
150 x 4.6 mm	1.74
100 x 4.6 mm	1.16
50 x 4.6 mm	0.58
250 x 4.0 mm	2.20
125 x 4.0 mm	1.10
250 x 2.0 mm	0.55
150 x 2.0 mm	0.33
50 x 2.0 mm	0.11

$CV = \pi r^2 L$

where:

CV = column volume in mL
r = column radius in cm
L = column length in cm

Table 2. Example Solution Volume Calculations

Mobile Phase solution		
Operation	***Calculation***	***Volume (ml)***
Prime and purge	20 mL	20
Increasing flow rate	0.75 mL/min * 5 min	4
Initial equilibration	15 CV = 15 * 3 mL	45
Assays	N * flow rate * run time = 4 * 0.75 mL/min * 15 min	45
Syringe cleaning	10 mL	10
Re-equilibration	N * 5 CV = 4 * 3 mL	12
Reserve volume	100 mL	100
Subtotal	Sum of the above	236
Minimum volume	Subtotal * 120%	**284**

Storage solution		
Operation	***Calculation***	***Volume (ml)***
Prime and purge	20 mL	20
Increasing flow rate	0.5 mL/min * 5 min	3
Wash	15 CV = 15 * 3 mL	45
Reserve volume	100 mL	100
Subtotal	Sum of the above	168

SOP: Buck Scientific BLC-30G HPLC Operation

Minimum volume	Subtotal * 120%	**202**

Equation 1. Example Run Time Calculations

$Run\ volume = 15\ CV$
$Column\ volume\ (CV) = 3\ mL$
$Flow\ rate = \ 0.75\ ml/min$

$$Run\ time = \frac{Run\ volume}{Flow\ rate} = \frac{15\ CV\ *\ 3\ mL/CV}{0.75\ mL/min} = 60\ min.$$

Table 3. Example Flow Rates & Pressure Readings for Ultra C8 5µm 250 X 4.6mm Column (Restek)

Mobile Phase Solution	***Flow Rate (mL/min)***	***Pressure Readings (psi)***
50% MeOH/H_2O	0.5	~1450

11. History

Revision Number	***Effective Date***	***Preparer***	***Description of Change***
0	17JUL15	John Buford, Jason McMillan, Jack O'Neill	Initial release

SOP: Characterization of Green Fluorescent Protein using BLC-30G HPLC System

Approvals

Preparer: John Buford, Jason McMillan, Jack O'Neill Date: 16JUL15
Reviewer: Dr. Maggie Bryans Date: 17JUL15

1. **Purpose**
   1.1. Characterization of Green Fluorescent Protein (GFP) in a sample using a Buck Scientific BLC-30G HPLC Gradient system. The GFP is detected using both UV-Vis and Fluorescence detectors.
2. **Scope and Applicability**
   2.1. High performance liquid chromatography (HPLC) is an analytical chemistry technique that separates the components of a liquid sample to assist in the identification and quantification of the components within the sample. This Process SOP makes use of reverse-phase HPLC to separate the constituents of a GFP sample on a Restek C8 column in a 50% MeOH/H_2O mobile phase. This SOP details the HPLC column selection, flow rates, operational run times, mobile phase and sample preparations to perform this separation.
3. **Summary of Method**
   3.1. Prepare a mobile phase solution of 50% MeOH/H_2O
   3.2. Power up the BLC-30G HPLC system and equilibrate the HPLC with the mobile phase
   3.3. Set the parameters for the detectors.
   3.4. Prepare the assay sample in the mobile phase.
   3.5. Set the assay conditions.
   3.6. Run an assay for each of the samples and record the data
   3.7. Wash the system with mobile phase solution
   3.8. Power down the system
4. **References**
   4.1. SOP: Degassing a Solution by Helium Sparge, document number QCB 6, revision 0, effective 25SEP13.
   4.2. SOP: Buck Scientific BLC-30G HPLC Operation, QCB 9, revision 0, effective 17JUL15
5. **Definitions**

CV	Column Volume; the volume (mL) of the column containing the stationary phase; CV=2.91 mL for a standard size (4.6 X 250 mm) column
Equilibration	Running the mobile phase solution through the column prior to injecting the sample in order to bring the system into equilibrium
Flow rate	The rate (mL/min) that liquid solution is pumped through the column. The operating flow rate is determined by the assay protocol.
Fluorescence detector, FL	A detector that measures the emitted light from the sample cell. The emitted light is of higher wavelength (lower in energy) than that of excitation wavelength.

SOP: Characterization of Green Fluorescent Protein using BLC-30G HPLC System

Gradient	The composition of the mobile phase is variable; the system has two pumps
Helium sparge	Using a stream of helium bubbles to sweep dissolved air out of liquids (helium is virtually insoluble in most HPLC solvent solutions, so very little helium replaces the air)
HPLC	High Performance Liquid Chromatography
Mobile phase	The solvent solution used to carry the sample through the column
PeakSimple	Software used to collect and display data
PSI	Pounds per Square Inch
Reverse Phase chromatography	Separation based on hydrophobicity under conditions where the stationary phase is more hydrophobic than the mobile phase.
Stationary phase	The chromatography matrix through which the sample travels.
UV-Vis detector	A detector that measures the % transmitted light across the sample cell and converts it to Absorbance, ABS. The detector wavelength is variable.

6. Precautions

6.1. Wear personal protection equipment (PPE) and use a fume hood as required.

6.2. Use HPLC-grade solvents and filter solutions with a sub-micron filter (preferably 0.22 μm). Degas solutions prior to use.

6.3. Methanol is flammable. Can cause blindness if swallowed. Vapor is harmful. Irritating to skin and eyes. Read the Material Safety Data Sheet (MSDS) for additional hazards, handling and storage information. Store solvents as indicated by the MSDSs.

6.4. HPLC systems operate at high pressures. Personnel injury and equipment damage can result if maximum pressure is exceeded or the pump runs dry. Monitor pressure readings and solution level whenever the pump is running. If pressure exceeds 2500 psi or if the solution runs out, stop the pump immediately by pressing the RUN/STOP button. Do not set the flow rate higher than 1.5 ml/min with a 250 mm column.

6.5. To avoid microbial growth, do not leave the system in a high aqueous solution for a prolonged period. The system should be washed with a storage solution of 50% Methanol/H_20 or 50% Acetonitrile/H_20 if it is to be idle more than a few hours.

7. Responsibilities

7.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.

7.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

8. Equipment and Materials

8.1. Buck Scientific BLC-30G HPLC system pre-configured with:

8.1.1. UV-Vis detector

8.1.2. Fluorescence Detector (optional)

SOP: Characterization of Green Fluorescent Protein using BLC-30G HPLC System

8.1.3. PeakSimple Chromatography Data System
8.1.4. Computer system with PeakSimple software installed
8.1.5. Restek Ultra C8 5µm 250 X 4.6mm HPLC column
8.2. Analytic balance
8.3. Assorted micropipettes
8.4. Green Fluorescent Protein sample
8.5. HPLC-grade methanol to prepare the mobile phase solution
8.6. HPLC-grade water to prepare the mobile phase solution
8.7. Nalgene Rapid-flow filtration unit with sub-micron filters (preferably 0.22 µm), that is chemically compatible with the mobile phase
8.8. Parafilm
8.9. Stirring plate
8.10. Timer
8.11. 2 – 50 mL laboratory beakers (for overflow waste and to rinse the sample syringe)
8.12. 2 – 500 mL laboratory bottles (for mobile phase solution and waste)
8.13. 4 – microfuge tubes (for GFP and diluted GFP samples)
8.14. 25 mL Luer-Lok syringe to purge the HPLC pumps
8.15. 100 µL HPLC sample syringe
8.16. 100 mL volumetric flask
8.17. 250 mL or 500 mL graduated cylinder
8.18. 500 mL volumetric flask

9. Procedure

9.1. Prepare 500 mL 50% methanol/H_2O mobile phase solution:
9.1.1. Measure 250 mL HPLC-grade methanol using a graduated cylinder and pour into a 500 mL volumetric flask.
9.1.2. Bring to volume 500 mL with HPLC-grade H_2O. Cover with parafilm and invert to mix. Check the volume and adjust as necessary (when methanol and water combine, the total volume may be slightly less than the original volumes).
9.1.3. Filter the mobile phase solution using a Nalgene Rapid-flow filtration unit. Transfer approximately 10 mL of mobile phase solution to a small labeled bottle to be used for rinsing the sample syringe. Transfer remaining solution to a labeled 500 mL laboratory bottle.
9.1.4. Sparge the mobile phase with Helium per the Degassing a Solution by Helium Sparge SOP.
9.1.5. Label an empty bottle as mobile phase solution waste.
9.2. Power up the HPLC system and equilibrate with mobile phase solution:
9.2.1. Power up the HPLC system components and start the PeakSimple data collection software per the Buck Scientific BLC-30G HPLC Operation SOP.
9.2.2. Switch the system to mobile phase solution per the Buck Scientific BLC-30G HPLC Operation SOP.
9.2.3. Purge the intake line and prime the pump with mobile phase solution per the Buck Scientific BLC-30G HPLC Operation SOP.

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9.2.4. Set the pumps to 50% Pump A and Pump B per the Buck Scientific BLC-30G HPLC Operational SOP and confirm that both Pumps A & B are set at 50%.

9.2.5. Gradually increase the flow rate to 0.5 mL/min over 5 minutes per the Buck Scientific BLC-30G HPLC Operation SOP. Monitor the pressure readings and verify that mobile phase solution is dripping into the waste bottle.

9.2.6. Equilibrate the system with mobile phase solution at the flow rate 0.5 mL/min for 30 minutes per the Buck Scientific BLC-30G HPLC Operational SOP.

9.3. Set the UV-Vis detector wavelength and autozero the detector:

9.3.1. Set the UV-Vis detector wavelength to 400 nm. After 60 minutes, autozero the detector per the Buck Scientific BLC-30G HPLC Operation SOP.

9.3.2. Ensure that the UV-Vis detector warms up for 60 minutes prior to collecting data per the Buck Scientific BLC-30G HPLC Operation SOP.

9.4. Prepare the GFP sample solutions:

9.4.1. Locate the 3.74 mg/mL GFP stock solution and confirm its use for this exercise.

9.4.2. Using micropipettes with the GFP stock, prepare 500 µL each of a 1-to-5 and 1-to-10 GFP sample dilutions (in mobile solution) in labeled microfuge tubes. Pipette up and down, then cap and vortex to mix each sample.

9.5. Set the assay conditions:

9.5.1. For each of the GFP samples, set up an assay for 8 minutes at a flow rate of 0.5 mL/min.

9.5.1.1. Use PeakSimple to start a new run.

9.5.1.2. Select File > New from the menu bar.

9.5.1.3. Select Edit > Channels… from the menu bar. The Channels dialog box should appear.

9.5.1.4. Next to Channel 1:Uv-Vis, ensure that “active,” “display” and “integrate” all have checks in them.

9.5.1.5. Select “Details”

9.5.1.6. Under the section “Control by” select “Gradient”

9.5.1.7. Under “End Time” input a value of 8 minutes for the length of the run.

9.5.1.8. Verify that the “Remote start” check box is checked

9.5.1.9. Press “OK” when finished.

9.5.1.10. Select “Gradient”

9.5.1.11. Select “Clear”

9.5.1.12. Select “Add”

9.5.1.13. Enter 50 into the % field for initial gradient.

9.5.1.14. Press “OK” when finished.

9.5.1.15. Close the dialog boxes for Channel 1 and repeat steps 9.5.1.4-9.5.1.9 for Channel 2.

9.5.1.16. Next to Channel 6, ensure that “active,” and “display” have checks in them.

9.5.1.17. Select “Details”

9.5.1.18. Ensure that “Datalogger mode” has a check in the box next to it.

SOP: Characterization of Green Fluorescent Protein using BLC-30G HPLC System

9.5.1.19. Ensure Offset = 0
9.5.1.20. Ensure Gain = 1
9.5.1.21. Ensure Decimal Places = 0
9.5.1.22. Under "End Time" input a value of 8 minutes for the length of the run.
9.5.1.23. Press "OK" when finished.
9.5.1.24. Press "OK" to close the Channels window.

9.6. For each sample, run an assay:
9.6.1. For each of the GFP samples, run an assay for 8 minutes at a flow rate of 0.5 mL/min.
9.6.1.1. Use PeakSimple to start a new 8 minute run.
9.6.1.2. Autozero the UV-Vis detector.
9.6.2. Load and inject a 100µL GFP sample using a syringe as per the Buck Scientific BLC-30G HPLC Operation SOP.
9.6.2.1.1. Verify that the injector port handle is set to the "Load" position.
9.6.2.1.2. Fill the HPLC sample syringe with 100 µL of sample, using care to avoid bubbles in the syringe.
9.6.2.1.3. Insert the syringe needle into the sample injection port (Figure 3).
9.6.2.1.4. Depress the syringe plunger, using care to avoid introducing bubbles. (Often there is a small bubble at the base of the plunger. Watch carefully and stop depressing the plunger before the bubble is loaded into the injector port. It is OK to leave a few µL of sample in the syringe.)
9.6.2.1.5. Turn the injector port handle clockwise from the "Load" to the "Inject" position (Figure 4). Turning the port will engage the PeakSimple software to START the assay and the elapsed run time will display in upper right corner. Leave the port in the "Inject" position.
9.6.2.1.6. After 10 seconds, turn the injector port handle counter-clockwise from the "Inject" position back to the "Load" position.
9.6.2.2. Remove the syringe from the sample injection port.
9.6.2.3. Rinse the syringe by filling it from the small bottle of mobile phase solution and expelling it into the mobile phase waste bottle at least three times.
9.6.2.4. Operate the pump for 8 minutes at 0.5 mL/min. At the end of the assay run, the PeakSimple elapsed time switches to STANDBY mode in the upper corner.

9.7. For each assay, view the results and collect/save the data:
9.7.1. Identify/save both the GFP peak/s of interest on the chromatographs and the retention times for each sample run.
9.7.1.1. Select View > Results… from the menu bar. The Results dialog box should appear.
9.7.1.2. Click the Copy button to copy the data.
9.7.1.3. Paste the data into an Excel spreadsheet.
9.7.1.4. Close the Results dialog box.
9.7.2. Select File > Save as… from the menu bar. The Save as dialog box should appear. Save the data to a separate chromatogram file.

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9.7.2.1. Enter a directory and a meaningful file name (e.g. operator initials, experiment name, and run number). Click the Save button.

9.8. Repeat assay.

9.8.1. Repeat another assay sample run as per 9.6 until each sample has been placed across the column

9.9. Wash the system with 5 CV of mobile phase as per Buck Scientific BLC-30G HPLC Operational SOP

10. Compute data for the GFP samples:

10.1.1. Document the retention time and peak heights of the GFP samples using Excel.

11. Stop the pump and allow the pressure to decrease to 0 per the Buck Scientific BLC-30G HPLC HPLC Operation SOP.

12. Power down the system per the Buck Scientific BLC-30G HPLC HPLC Operational SOP.

13. Attachments

Figure 1. Buck Scientific BLC-20G HPLC System with Pumps and Detectors

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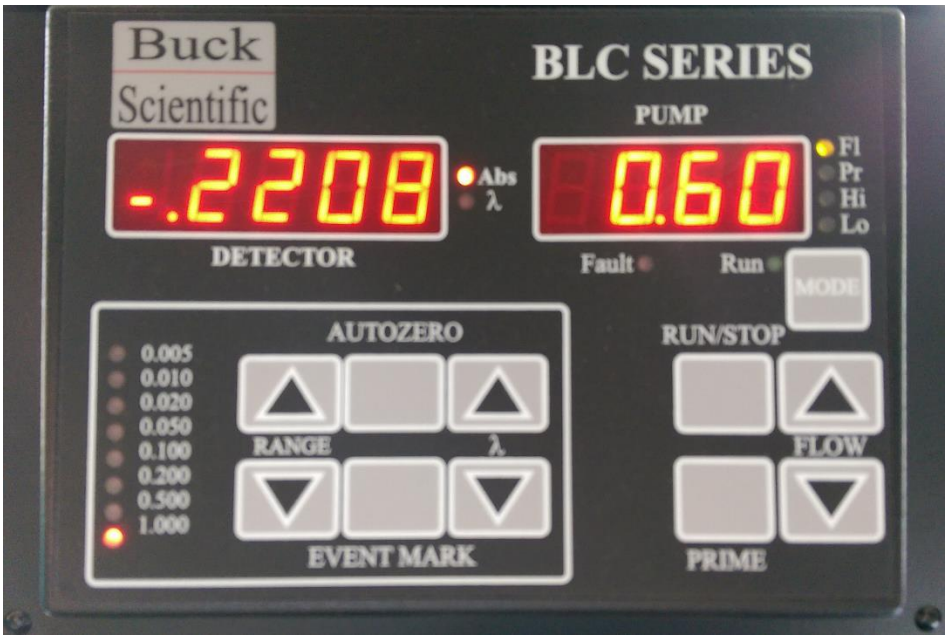


Figure 2. HPLC Front Panel

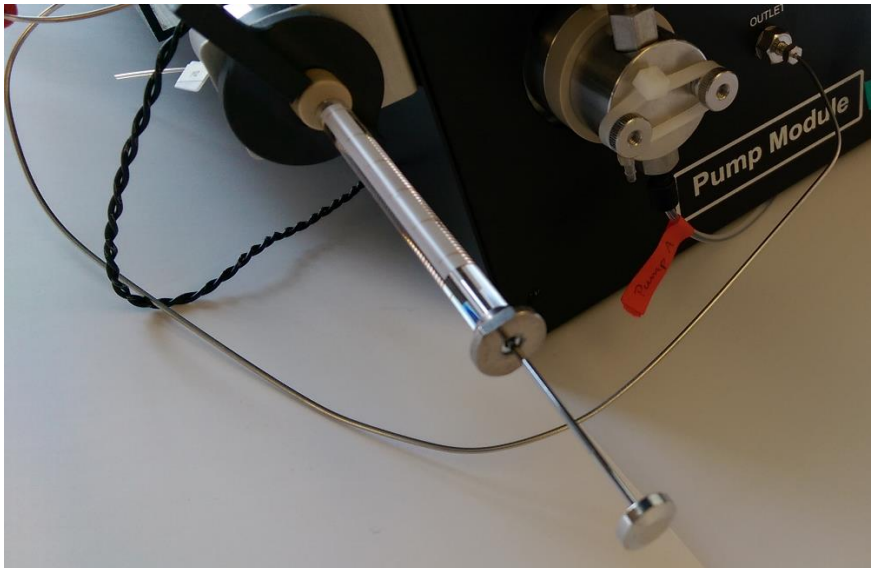
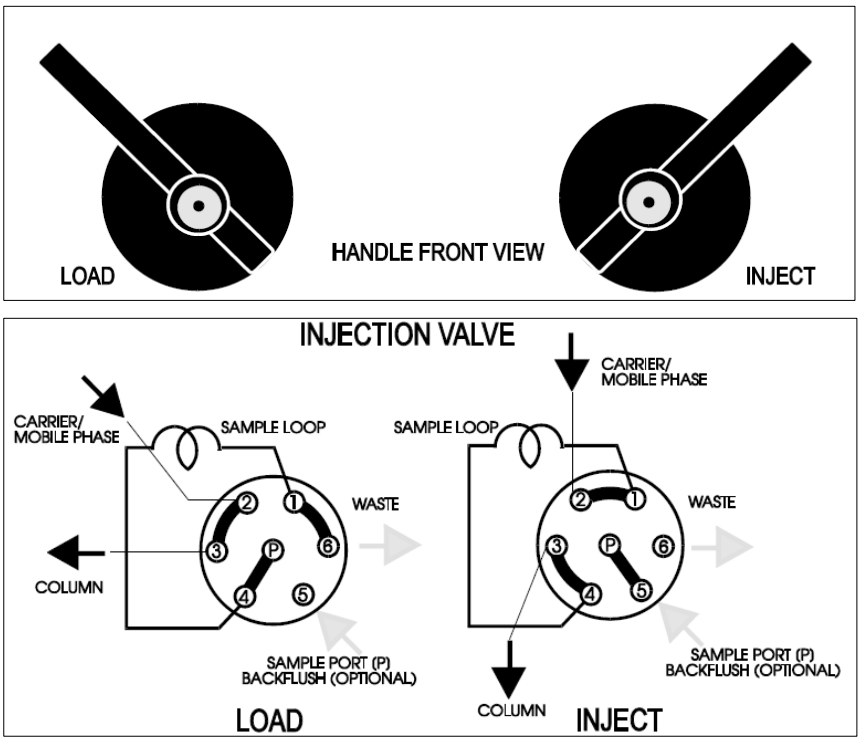


Figure 3. HLPC Syringe in the Sample Injection Port in the 'Load' Position

SOP: Characterization of Green Fluorescent Protein using BLC-30G HPLC System



Figures 4. Sample Injection Port Positions: 'Load' and 'Inject'

14. History

Revision Number	*Effective Date*	*Preparer*	*Description of Change*
0	17JUL15	John Buford, Jason McMillan, Jack O'Neill	Initial release

SOP: Operation of NanoDrop 2000 Spectrophotometer

Approvals

Preparer: John Buford Date: 24OCT13
Reviewer: Jason McMillan Date: 24OCT13
Reviewer: Jack O'Neill Date: 24OCT13

1. **Purpose**
   1.1. Measure the light absorbance of microliter (µL) samples using a NanoDrop 2000 spectrophotometer in order to analyze ultraviolet and visible light spectral signature and to quantify DNA and protein content based on spectral signature.
2. **Scope and Applicability**
   2.1. A spectrophotometer transmits known wavelengths of light through a sample and measure the amount of light absorbed by the sample. Nucleic acids (such as DNA) and proteins absorb light at the 260 nm and 280 nm respectively, so a spectrophotometer can be used to quantify the DNA and protein content of a sample. This SOP describes the general operation of the NanoDrop 2000 spectrophotometer and its use to measure DNA concentration, protein concentration, and ultraviolet-visible (UV-Vis) light absorption.
3. **Summary of Method**
   3.1. Connect and power up the spectrophotometer to a PC.
   3.2. Launch the NanoDrop 2000 software interface.
   3.3. Apply 2 µL blank solution onto the lower pedestal and initiate a blank measurement.
   3.4. Wipe the blank from both the upper and lower pedestals using a clean lint-free laboratory wipe.
   3.5. Apply 2 µL sample solution onto the lower pedestal and initiate a spectral measurement.
   3.6. Wipe the sample from both the upper and lower pedestals using a clean lint-free laboratory wipe.
4. **References**
   4.1. Thermo Scientific NanoDrop 2000/2000c Spectrophotometer V1.0 User Manual
5. **Definitions**

Spectrophotometer	an instrument for measuring light intensity of known light wavelengths
UV-Vis	Ultraviolet and visible light

6. **Precautions**
   6.1. The NanoDrop 2000 spectrophotometer contains no user serviceable parts; do not open.
7. **Responsibilities**
   7.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   7.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
8. **Equipment and Materials**
   8.1. NanoDrop 2000 spectrophotometer
   8.2. 12V power supply

SOP: Operation of NanoDrop 2000 Spectrophotometer

8.3. PC with NanoDrop 2000/2000c software interface installed
8.4. Precision micropipette (2 µL or 10 µL) and micropipette tips
8.5. Laboratory grade water (dH_2O)
8.6. Sample solution(s)
8.7. Blank solution
8.8. Lint-free laboratory wipes (e.g. Kimwipes)

9. Procedure

9.1. ***Connecting and powering up the spectrophotometer***

9.1.1. Connect the USB cable to the NanoDrop 2000 spectrophotometer.
9.1.2. Plug in the 12V power supply and connect to the power input at the back of the spectrophotometer. (The power supply may remain plugged into the spectrophotometer while not in use.)

9.2. ***Launching the NanoDrop 2000 software interface***

9.2.1. Double click the NanoDrop 2000 icon of the desktop.
9.2.2. Alternatively, select Start > All Programs > Thermo Scientific NanoDrop 2000 > NanoDrop 2000.
9.2.3. The Home window should appear, containing task options on the left pane (e.g. Home, My Data, Diagnostics) and application buttons on the right pane (e.g. Nucleic Acid, Protein A280, UV-Vis).
9.2.4. Selecting a task option or application replaces the Home window with a window for that specific task or application.
9.2.5. To return to the Home window from any other window, select the "Home" task option from the left pane.

9.3. ***Basic use of the pedestal***

9.3.1. Raise the sampling arm.
9.3.2. Apply 2 µL solution onto the lower pedestal using a micropipette.
9.3.3. Lower the sampling arm.
9.3.4. Initiate a spectral measurement using the PC software interface.
9.3.5. When measurement is complete, raise the sampling arm and wipe the solution from both the upper and lower pedestals using a clean lint-free laboratory wipe.
9.3.6. Lower the sampling arm.
9.3.7. Leave the sampling arm in the down position between uses.

9.4. ***Pedestal cleaning***

9.4.1. Use dH_2O to clean the measurement surfaces before the first sample measurement and after the last sample measurement.
9.4.2. Raise the sampling arm.
9.4.3. Apply 3-5 µL dH_2O onto the lower pedestal using a micropipette.
9.4.4. Lower the sampling arm.
9.4.5. Let sit for approximately 2-3 minutes.
9.4.6. Raise the sampling arm and wipe the dH_2O from both the upper and lower pedestals using a clean lint-free laboratory wipe.
9.4.7. Lower the sampling arm.
9.4.8. Generally, it is not necessary to clean with dH_2O between samples.

9.5. ***Performing an intensity check***

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9.5.1. At regular intervals (weekly or monthly), confirm that the internal spectrometer is functioning as expected.
9.5.2. Select the "Diagnostic" task from the Home window.
9.5.3. Select the "Intensity Check" task.
9.5.4. Ensure that the sampling arm is down.
9.5.5. Click "Measure".
9.5.6. If yellow triangles are displayed in the top left of the spectral display, clean the pedestal and then repeat the intensity check.
9.5.7. Return to the Home window by selecting the "Home" task option from the left pane.

9.6. ***Measuring DNA concentration***

9.6.1. Select the "Nucleic Acid" application from the Home window.
9.6.2. If the wavelength verification window appears, ensure that the sampling arm is down and click OK.
9.6.3. Select File > New Workbook, enter a filename and directory, then click Save.
9.6.3.1.(Optional) Select the type of sample to be measured from the "Type" drop-down list (the default is DNA-50).
9.6.4. Select the "Add to report" checkbox to include measurements in the current report.
9.6.5. Select the "Overlay spectra" checkbox if more than one sample is to be measured and displayed on the same spectral graph.
9.6.6. Establish a blank using the appropriate blank solution:
9.6.6.1.Apply 2 µL blank solution onto the lower pedestal per *9.3 Basic use of the pedestal*.
9.6.6.2.Click the "Blank" button.
9.6.6.3. When the blank measurement is complete, wipe the blank solution from both the upper and lower pedestals using a clean lint-free laboratory wipe per *9.3 Basic use of the pedestal*.
9.6.7. Measure each sample:
9.6.7.1.Enter a sample ID in the "Sample ID" field.
9.6.7.2.Apply 2 µL sample solution onto the lower pedestal per *9.3 Basic use of the pedestal*.
9.6.7.3.Click the "Measure" button.
9.6.7.4. When the sample measurement is complete, wipe the sample solution from both the upper and lower pedestals per *9.3 Basic use of the pedestal*.
9.6.7.5.Repeat with the next sample solution if more than one sample is to be measured and displayed on the same spectral graph.
9.6.8. Click the "Print" button to print the spectral display. Alternatively, select the Reports task option to format and print a report.
9.6.9. Save the data and return to the Home window by selecting File > Close Workbook and go Home.
9.6.10. After the last sample of the day, clean the pedestal per *9.4 Pedestal cleaning*.

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9.7. ***Measuring protein concentration***

9.7.1. Select the "Protein A280" application from the Home window.

9.7.2. If the wavelength verification window appears, ensure that the sampling arm is down and click OK.

9.7.3. Select File > New Workbook, enter a filename and directory, then click Save.

9.7.3.1.(Optional) Select the type of sample to be measured from the "Type" drop-down list (the default is 1 Abs = 1 mg/mL).

9.7.4. Select the "Add to report" checkbox to include measurements in the current report.

9.7.5. Select the "Overlay spectra" checkbox if more than one sample is to be measured and displayed on the same spectral graph.

9.7.6. Establish a blank using the appropriate blank solution:

9.7.6.1.Apply 2 µL blank solution onto the lower pedestal per *9.3 Basic use of the pedestal*.

9.7.6.2.Click the "Blank" button.

9.7.6.3. When the blank measurement is complete, wipe the blank solution from both the upper and lower pedestals using a clean lint-free laboratory wipe per *9.3 Basic use of the pedestal*.

9.7.7. Measure each sample:

9.7.7.1.Enter a sample ID in the "Sample ID" field.

9.7.7.2.Apply 2 µL sample solution onto the lower pedestal per *9.3 Basic use of the pedestal*.

9.7.7.3.Click the "Measure" button.

9.7.7.4. When the sample measurement is complete, wipe the sample solution from both the upper and lower pedestals per *9.3 Basic use of the pedestal*.

9.7.7.5.Repeat with the next sample solution if more than one sample is to be measured and displayed on the same spectral graph.

9.7.8. Click the "Print" button to print the spectral display. Alternatively, select the Reports task option to format and print a report.

9.7.9. Save the data and return to the Home window by selecting File > Close Workbook and go Home.

9.7.10. After the last sample of the day, clean the pedestal per *9.4 Pedestal cleaning*.

9.8. ***Measuring UV-Vis absorbance***

9.8.1. Select the "UV-Vis" application from the Home window.

9.8.2. If the wavelength verification window appears, ensure that the sampling arm is down and click OK.

9.8.3. Select File > New Workbook, enter a filename and directory, then click Save.

9.8.4. Enter up to 40 wavelengths of interest in the "nm – Add wavelength(s)" list:

9.8.4.1.Select the first row, enter a wavelength (in nm), then press the Entry key.

9.8.4.2.Continue entering values until all wavelengths of interest have been added.

9.8.4.3.A wavelength can be cleared by selecting the wavelength, then clicking the "Clear Wavelength" button.

9.8.5. Select the "Add to report" checkbox to include measurements in the current report.

SOP: Operation of NanoDrop 2000 Spectrophotometer

9.8.6. Select the "Overlay spectra" checkbox if more than one sample is to be measured and displayed on the same spectral graph.

9.8.7. Establish a blank using the appropriate blank solution:

9.8.7.1. Apply 2 µL blank solution onto the lower pedestal per *9.3 Basic use of the pedestal*.

9.8.7.2. Click the "Blank" button.

9.8.7.3. When the blank measurement is complete, wipe the blank solution from both the upper and lower pedestals using a clean lint-free laboratory wipe per *9.3 Basic use of the pedestal*.

9.8.8. Measure each sample:

9.8.8.1. Enter a sample ID in the "Sample ID" field.

9.8.8.2. Apply 2 µL sample solution onto the lower pedestal per *9.3 Basic use of the pedestal*.

9.8.8.3. Click the "Measure" button.

9.8.8.4. When the sample measurement is complete, wipe the sample solution from both the upper and lower pedestals per *9.3 Basic use of the pedestal*.

9.8.8.5. Repeat with the next sample solution if more than one sample is to be measured and displayed on the same spectral graph.

9.8.9. Click the "Print" button to print the spectral display. Alternatively, select the Reports task option to format and print a report.

9.8.10. Save the data and return to the Home window by selecting File > Close Workbook and go Home.

9.8.11. After the last sample of the day, clean the pedestal per *9.4 Pedestal cleaning*.

SOP: Operation of NanoDrop 2000 Spectrophotometer

10. Attachments

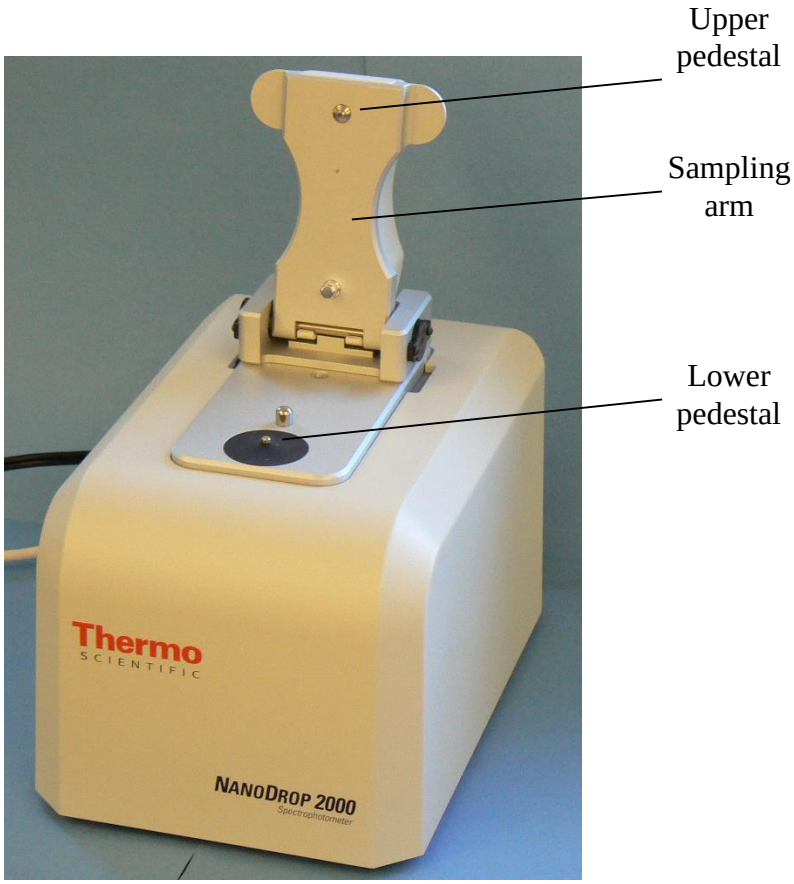


Figure 1. NanoDrop 2000 Spectrophotometer

SOP: Operation of NanoDrop 2000 Spectrophotometer

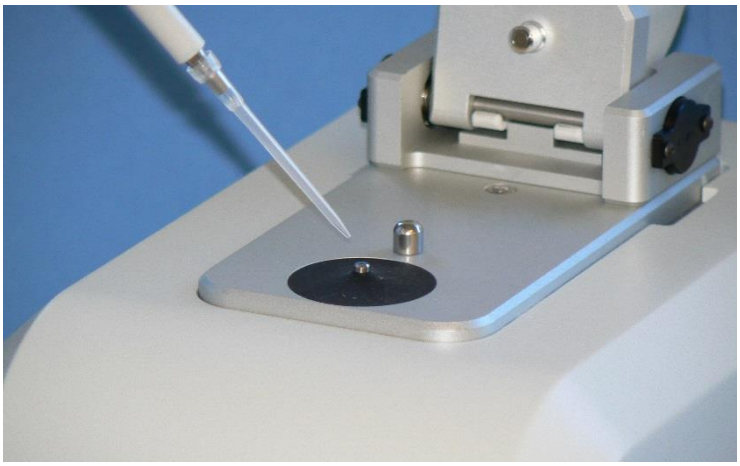
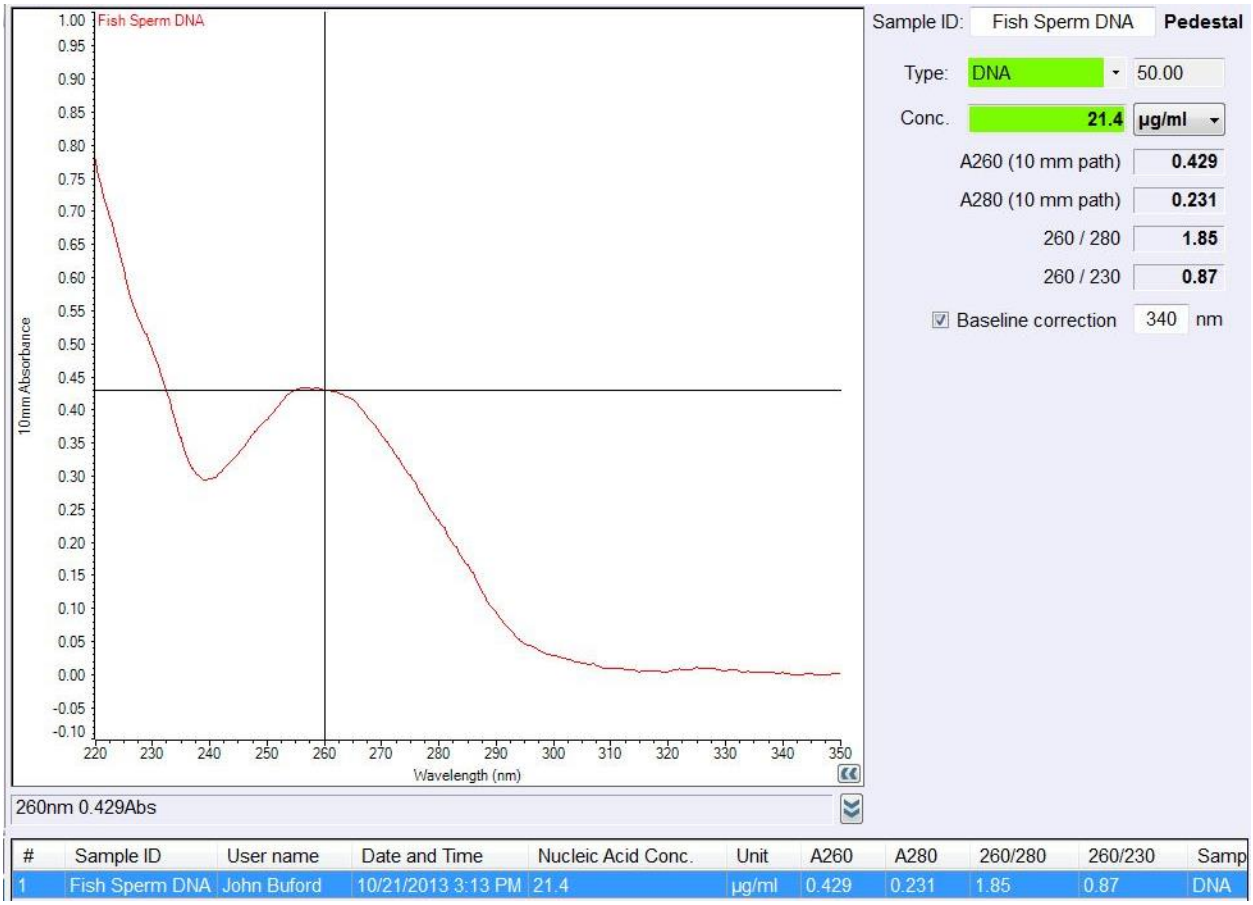


Figure 2. Applying solution onto the lower pedestal



SOP: Operation of NanoDrop 2000 Spectrophotometer

Figure 3. DNA Concentration Spectral Graph

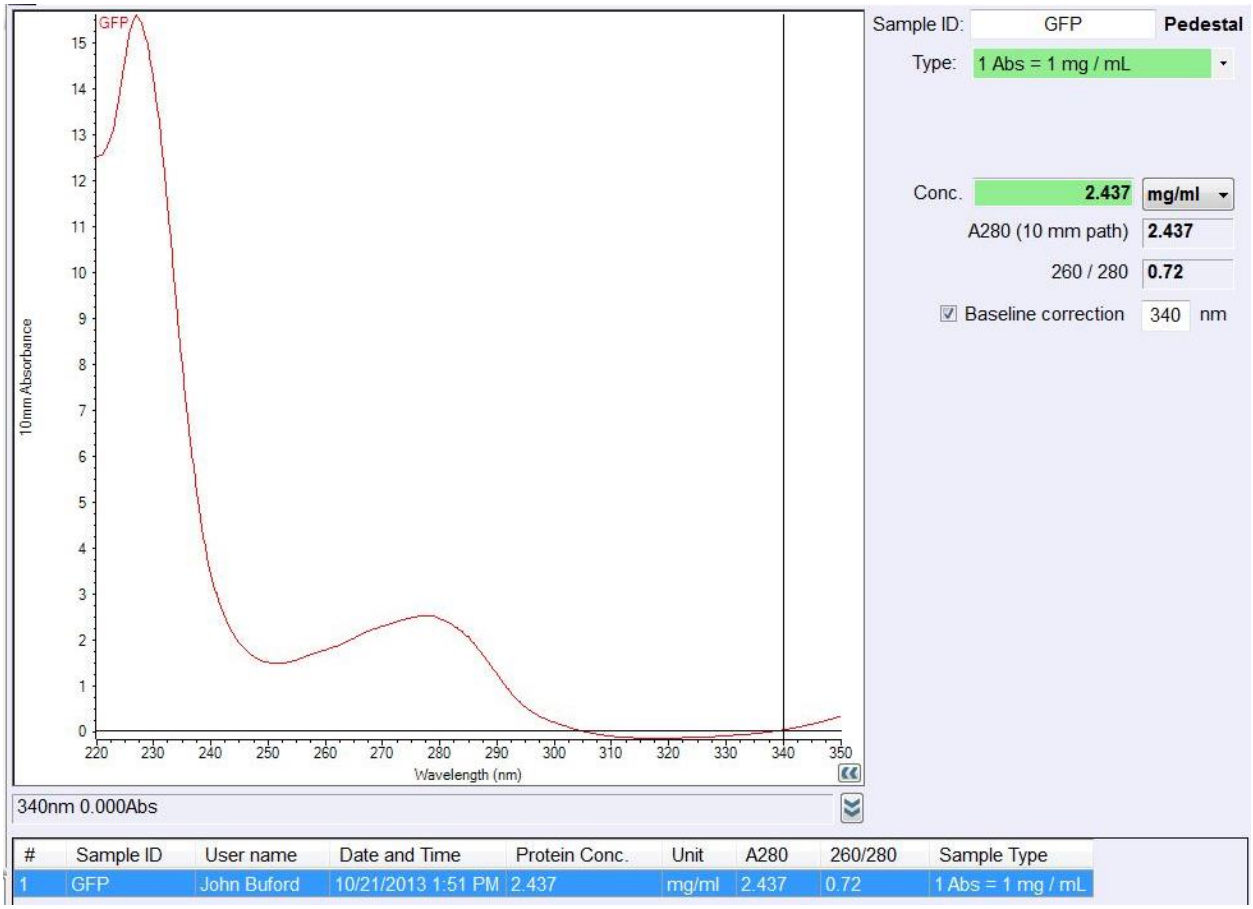
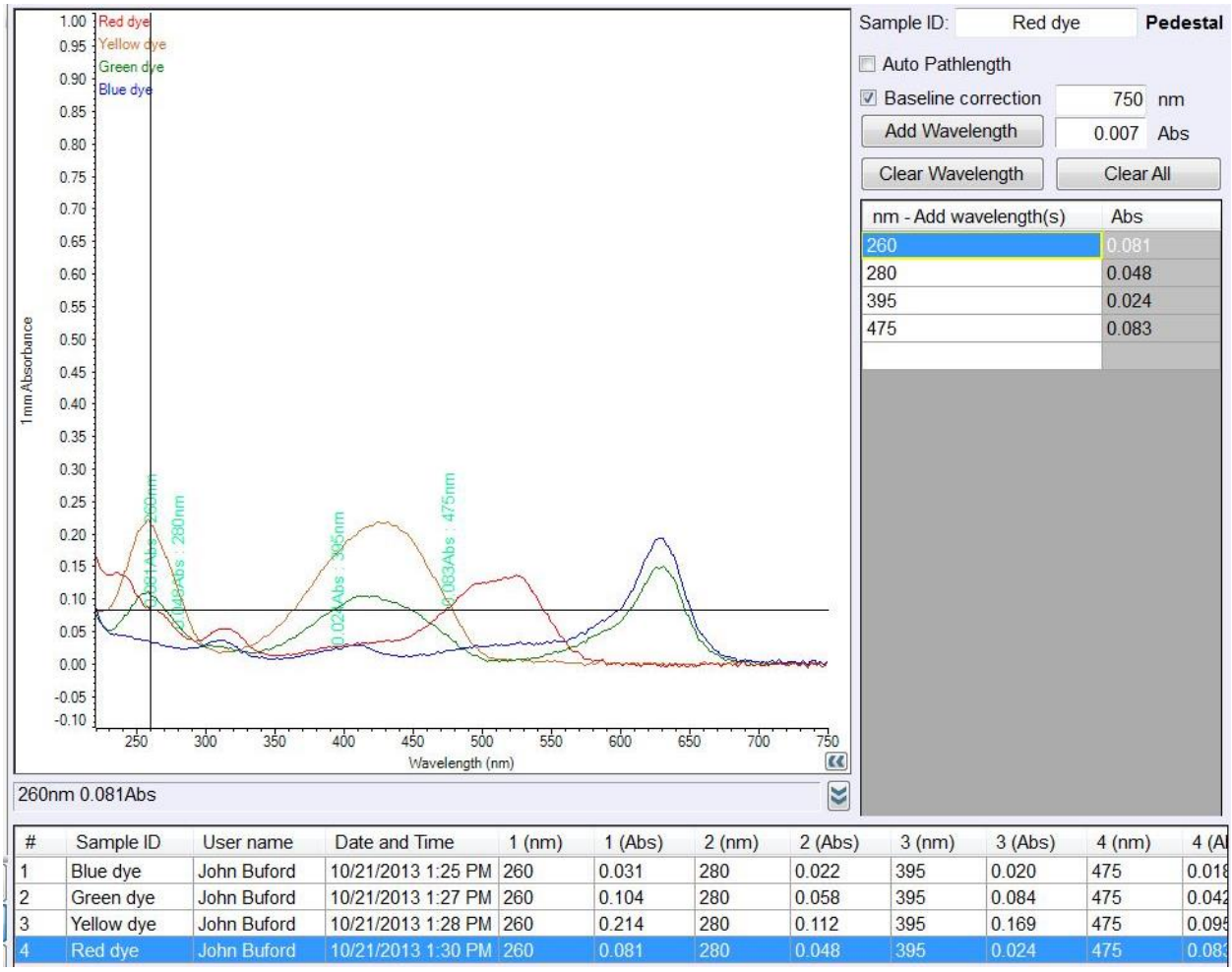


Figure 4. Protein Concentration Spectral Graph

SOP: Operation of NanoDrop 2000 Spectrophotometer



#	Sample ID	User name	Date and Time	1 (nm)	1 (Abs)	2 (nm)	2 (Abs)	3 (nm)	3 (Abs)	4 (nm)	4 (Al
1	Blue dye	John Buford	10/21/2013 1:25 PM	260	0.031	280	0.022	395	0.020	475	0.018
2	Green dye	John Buford	10/21/2013 1:27 PM	260	0.104	280	0.058	395	0.084	475	0.042
3	Yellow dye	John Buford	10/21/2013 1:28 PM	260	0.214	280	0.112	395	0.169	475	0.095
4	Red dye	John Buford	10/21/2013 1:30 PM	260	0.081	280	0.048	395	0.024	475	0.083

Figure 5. UV-Vis Spectral Graph

11. History

Revision Number	*Effective Date*	*Preparer*	*Description of Change*
0	10/24/2013	John Buford	Initial release