FOREWORD

This first publication of the Northeast Biomanufacturing Center and Collaborative (NBC_2) Global Biomanufacturing Curriculum (GBC) is a Core Production System utilizing Chinese Hamster Ovary (CHO) cells producing recombinant human tissue Plasminogen Activator (tPA). The Core Production System includes Upstream Processing (Cell Culture) Standard Operating Procedures (SOPs) with Batch Record, Downstream Processing (Purification) SOPs with Batch Record, and Quality Control Biochemistry SOPs, including SDS-PAGE, tPA ELISA, and a tPA Activity Assay.

tPA was the first protein to be commercially produced in CHO cells in 1987. In 1982, *Escherichia coli*, was used to commercially produce the first biopharmaceutical, recombinant human insulin. The problem with growing proteins in *Escherichia coli* is that they are not secreted by the cell so downstream processing or purification is quite difficult. Today, mammalian cells, particularly CHO cells, are the cells of choice for biopharmaceutical production because they secrete the protein biopharmaceutical. This is the reason we start with the publication of the CHO-tPA Core Production System. Other Core Production Systems are available as part of the GBC and still others are in development, utilizing *Escherichia coli* and *Pichia pastoris*.

The construction of the CHO Cell-tPA Standard Operating Procedures in this publication began in 1994 with a grant from the National Science Foundation Advanced Technological Education program's first solicitation in 1993. Many people have worked on the development of these SOPs, including Dr. Sonia Wallman from Great Bay Community College* (GBCC), the Principal Investigator on the NSF ATE grant awarded in 1994 and Karen Bresciano, a student technician who worked for Dr. Wallman as she pursued her Biotechnology Associate in Science degree at GBCC from 1995-1997.

In 2004, GBCC was one of five grantees to receive funding from the Department of Labor's High Job Growth Training Initiative to form the National Center for the Biotechnology Workforce. This grant allowed us to purchase Applikon 3 liter bioreactors, Bio-Rad Liquid Chromatography systems, and a Microplate Reader so we were able to vastly improve the upstream and downstream processing SOPs and to add a number of Quality Control Biochemistry SOPs . Three people were key to the final development of the CHO-tPA Core Production System: Deb Audino, Professor of Biotechnology/Biomanufacturing; Robert O'Brien, Biomanufacturing Laboratory Manager; and Kari Britt, Laboratory Technician, all in the Biotechnology Department at GBCC.

We wish to recognize the Department of Labor's Employment and Training Administration and the National Science Foundation's Advanced Technological Education Program (NSF #0501953) for their contribution to the development of this set of Standard Operating Procedures to support biomanufacturing technician education and training and the biomanufacturing workforce.

*Formerly New Hampshire Community Technical College

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CHO Cell - tPA
CORE PRODUCTION SYSTEM

NSF NBC² Global Biomanufacturing Curriculum

Title: Batch Culture of Recombinant tPA Secreting CHO Cells SOP

Approvals:
Preparer:___Deb Audino_____Date _____04Apr08_____
Reviewer:__Bob O'Brien_____ Date _____04Apr08_____

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. 100mL Spinner Flask SOP
   4.3. Biological Safety Cabinet SOP
   4.4. CO_2 Incubator SOP
   4.5. pH Meter SOP
   4.6. spectrophotometer SOP
   4.7. Biolyzer SOP
   4.8. Biolyzer Pipet SOP
   4.9. Trypan Blue Assay SOP
   4.10. tPA ELISA SOP
   4.11. tPA Activity Assay SOP
   4.12. Applikon Bioreactor 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. ProCHO4 media (manufactured by Cambrex/Biowhittaker)
   7.9. 200mM glutamine
   7.10. 10mg/mL gentamycin
   7.11. sterile 100mL Bellco spinner flasks

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7.12. sterile transfer pipets (50mL, 10mL, and 2mL) and pipettor
7.13. CO_2 incubator containing magnetic stir plate
7.14. UV-visible recording spectrophotometer
7.15. cuvettes for spectrophotometer
7.16. 1.5mL microfuge tubes
7.17. microscope with 1000x magnification
7.18. cryogenic vials (1mL capacity) for storage of CHO cell master/working cell bank
7.19. sterile 250mL glass bottles for storage of CHO cell media
7.20. computer and Microsoft Excel for Windows
7.21. 100 mL glass bottle
7.22. Sigma 2K15 refrigerated centrifuge
7.23. biolyzer
7.24. biolyzer pipet

8. Procedure:

8.1. **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:
automatic pipettor
10mL sterile pipettes
50mL sterile pipettes
8.1.3. Prepare biological safety cabinet (BSC) per SOP.
8.1.4. Spray the outside of the following with 70% isopropanol then place in the biological safety cabinet:
100mL sterile Bellco spinner flasks
500mL bottles of pre-sterilized Ham's F12 Medium
100mL of pre-sterilized, heat inactivated fetal bovine serum (FBS)
8.1.5. Sterilely remove 90mL ± 1mL of Ham's F12 Medium from a 500mL bottle of Ham's F12 and place in a sterile 100mL spinner flask.
8.1.5.1. Repeat with a second 100mL spinner flask.
8.1.6. Sterilely add 10mL ± 1 of FBS to the Ham's F12 bottle.
8.1.6.1. Repeat with the second 100mL spinner flask.
8.1.7. Label all spinner flasks as 90% Ham's F12, 10% FBS, [date], [group#], [operator initials].
8.1.8. Place all 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 mixing of the culture without foaming.
8.1.8.1. Verify that the temperature is 37 ± 0.5°C and percentage of CO_2 is 5 ± 0.5%.
8.1.9. Check media for contamination after a minimum of 24 hours.
8.1.10. Store media in refrigerator.

8.2. **Inoculation**

8.2.1. Pre-warm the spinner flasks containing CHO cell culture medium at 37° C ± 0.5°C.

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8.2.2. Spray a minimum of four 2mL sterile pipettes with 70% isopropanol and place in the BSC.
8.2.3. Prepare Biological Safety Cabinet per BSC SOP.
8.2.4. Remove 2 vials of CHO cells from storage in the -86°C freezer.
8.2.5. Thaw contents rapidly by agitation in a 37°C ± 0.5°C water bath (Belly Dancer).
8.2.6. Spray vials with 70% isopropanol, and place in the biological safety cabinet.
8.2.7. Sterilely transfer the entire contents of each 1mL vial of thawed CHO Cells into each of the previously prepared Bellco Spinner Flask containing 100mL CHO Cell Culture Medium using a 2mL sterile pipet.
8.2.8. Swirl to mix.
8.2.9. Immediately after adding CHO Cells to Bellco spinner flask (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. Collect the following items, spray with 70% IPA and place in Biological Safety Cabinet:
2 microfuge tubes labeled "tPA, Tn" and "cells"
1 microfuge tube holder
3 spectrophotometers cuvettes
1 cuvette holder
4 2mL pipets
pipet pump
8.3.2. Prepare biological safety cabinet per BSC SOP.
8.3.3. Prepare pH Meter per pH Meter SOP.
8.3.4. Prepare biolyzer and biolyzer pipet per biolyzer and biolyzer pipet SOPs.
8.3.4.1. Remove lactate and glucose Biolyzer slides from the -20° C freezer.
8.3.5. Prepare spectrophotometer per spectrophotometer SOP using media to zero the machine.
8.3.6. Spray blank and culture spinner bottle with 70% IPA and place in biological safety cabinet.
8.3.7. Using aseptic technique, remove 2 – 2.2mL sample of culture and place into a cuvette. Note: Do not mix blank and sample cuvettes.
8.3.8. Remove all items from the biological safety cabinet.
8.3.9. Return suspension culture and blank to the CO_2 incubator, making sure to loosen caps once in incubator. Set the speed of the magnetic stirrer to the maximum setting that ensures an even vortexing of the culture without foaming.
8.3.10. Cover the sample cuvette with parafilm.
8.3.11. Take OD Reading at 650nm per spectrophotometer SOP.
8.3.11.1. Mix CHO sample by inverting the cuvette several times before taking reading.
8.3.12. Take readings for glucose and lactate using the Biolyzer per the Biolyzer SOP.
8.3.13. Determine cell count using the Trypan Blue SOP.

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8.3.14. Take pH reading per pH meter SOP.
8.3.14.1. Transfer the sample to a test tube to measure the pH per the pH meter SOP.
8.3.15. Remove the sample to a 1.5mL tube and centrifuge at high speed for 5minutes. Remove the supernatant and store at 2-8°C until needed.

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

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

8.4.1. Prepare 1M $NaHCO_3$ (sodium bicarbonate)
8.4.1.1. Weigh out 21 ± 1 grams of $NaHCO_3$ and transfer to an Applikon bioreactor feed bottle.
8.4.1.2. Label the bottle as 1M $NaHCO_3$, [date], [initials], [group number], storage: room temp, disposal: drain.
8.4.1.3. Using a 250mL graduated cylinder, measure 250 ± 5mL deionized water and transfer into the feed bottle.
8.4.1.4. Add a magnetic stir bar and stir on a magnetic stirrer to dissolve.
8.4.2. Prepare 1X PBS.
8.4.2.1. Using a 10mL pipet, measure 10 ± 0.5mL of 10x PBS and dispense into a 100mL vessel.
8.4.2.2. Using a 100mL graduated cylinder, measure 90 ± 5mL of deionized water and transfer into the 100mL vessel. Swirl to mix.
8.4.2.3. Label vessel as 1X PBS, [date], [initials], [group number], storage: room temp, disposal: drain.
8.4.3. Set up Applikon bioreactors per the bioreactor SOP including calibrating the pH probe.
8.4.4. Autoclave the bioreactors with 1X PBS per the bioreactor SOP for 20 minutes.
8.4.5. Remove the bioreactor vessel from the autoclave and connect the DO probe to the controller.
8.4.6. Turn on the controller.
8.4.7. Allow the DO probe to polarize for a minimum of 6 hours.
8.4.8. In the biological safety cabinet prepare the media.
8.4.8.1. Add ~6mL of 200mM glutamine (1.2mM final concentration) and 10mL of 10mg/mL gentamycin (0.1mg/mL final concentration) to the 1L bottle of ProCHO4 media.
8.4.9. Aseptically pour the media into the bioreactor through the inoculation port using a sterile funnel.
8.4.10. Connect the remaining parts of the bioreactor to the controller.
8.4.11. Input the setpoints and limits listed in the table below per the bioreactor SOP.

Parameter	pH	Temp (^oC)	%DO	Stirrer (rpm)
Setpoint	7.2	37	50	75
Upper Limit	7.3	38	52	76
Lower Limit	7.1	36	48	74

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8.4.12. When the 100mL suspension culture of CHO cells reaches a concentration of approximately 1,000,000 cells/mL, aseptically transfer the culture to the bioreactor.
8.4.13. Start BioXpert Lite per bioreactor SOP.
8.4.14. Immediately and at 2-day intervals, sample the culture to determine OD, pH, viable cell count, analytes and tPA over time (see step 8.3).

8.5. When the cell count reaches approximately 1,000,000 cells/mL shut down the bioreactor per the bioreactor SOP and harvest cells as described below.

8.6. **Harvest and Preparation of Working Cell Bank**

8.6.1. Gather the following, spray with 70% IPA and place in the BSC:
30mL sterile centrifuge tubes (40)
25mL sterile pipets (10)
2mL sterile pipets (20)
cryovials (55)
250mL sterile glass bottles (4)
8.6.2. Prepare the biological safety cabinet per SOP.
8.6.3. Using a 25mL sterile pipet, transfer 25mL of the culture into sterile 30mL centrifuge tubes.
8.6.4. Centrifuge tubes for 10min. at 3000xg per centrifuge SOP.
Note: Always balance the test tubes in the centrifuge.
8.6.5. Prepare storage menstrum
8.6.5.1. Combine the following item into a container capable of holding >50mL and mix well.
40mL± 1.0mL of Ham's F12
5mL± 0.5mL of FBS
5mL± 0.5mL of glycerol
8.6.5.2. Filter sterilize.
8.6.5.3. Label bottle as CHO storage menstrum with the date.
8.6.5.4. Spray with 70% IPA and place in the biological safety cabinet.
8.6.5.5. Remove filter unit and place cap on bottle.
8.6.6. Following centrifugation of the culture, decant tPA containing medium into sterile 250mL bottles.
8.6.7. Label bottles as unpurified tPA in ProCHO4 [date} and [group #].
8.6.8. Store in the refrigerator at 2-8°C.
8.6.9. Add about 1mL of storage menstrum to each centrifuge tube to resuspend the pelleted CHO cells.
8.6.10. Sterilely dispense 1mL± 0.1mL aliquots into sterile 1.5mL cryovials.
50 cryovials for the working cell bank are expected.
8.6.11. Label in the following manner using a cryopen: CHO (ATCC CRL-9606) BT220-[day or evening], DATE.
8.6.12. Place in a styrofoam tube rack. Label container same as cryovials.
8.6.13. Store at -85°C.

8.7. **Determine tPA Concentration**

8.7.1. Determine the tPA concentration at each time point per tPA ELISA SOP.

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8.7.2. Determine the activity of the tPA at each time point per tPA Activity Assay SOP.

8.8. **Prepare Growth Curves**

8.8.1. Plot OD, pH, viable cells, glucose, lactate, and tPA vs. time (use 2 y-axes).

8.8.2. Attach growth curve to Batch Record.

8.8.3. Determine growth rate and doubling time of the 100mL spinner flask and 1L bioreactor cultures.

8.8.4. Attach calculations to Batch Record.

9. Attachments:

9.1. Data table

10. History:

Name	Date	Amendment
Sonia Wallman	2000	Initial Release
Deb Audino Ellery Raitt	6/2005	Put into 2005 SOP format. Removed trypan blue section and replaced with reference to Trypan Blue SOP. Added bioreactor section
Deb Audino	12Jan06	Removed 50mL and 500mL spinner flasks and replaced with 100mL spinner flasks. Reduce volume of storage menstrum added to cells from 2.5mL to 1mL
Deb Audino	05Mar08	Moved the polarization of the DO probe earlier in the procedure. Removed placing the bioreactor vessel in the BSC. Removed determne tPA activity.
Deb Audino	04Apr08	College name change

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

Title: Applikon Bioreactor Operation SOP

Approvals:
Preparer: ____Bob O'Brien_____Date _____03Apr08_____
Reviewer: ___Deb Audino_____ Date _____03Apr08_____

1. **Purpose:**
   1.1. Operation of the Applikon 3L Benchtop Bioreactor.
2. **Scope:**
   2.1. Applies to growing mammalian or insect cells in the Applikon 3L Benchtop Bioreactor.
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. Applikon Benchtop Bioreactor manufacturer instrumentation manuals
   4.2. autoclave SOP
   4.3. biological safety cabinet SOP
5. **Definitions:** N/A
6. **Precautions:**
   6.1. The sodium bicarbonate solution is very basic/caustic. Prevent contact with skin.
   6.2. The O_2 electrolyte used in the DO probe is a strong Alkaline (pH13) solution. Avoid contact with skin, mucous membrane, or eyes. If contact does occur flush effected area with water.
   6.3. The heat blanket is very warm when in use. Use caution.
7. **Materials :**
   7.1. pH 4.0 buffer standard
   7.2. pH 7.0 buffer standard
   7.3. lab towels
   7.4. 1X PBS
   7.5. 1M sodium bicarbonate
   7.6. silcone tubing
   7.7. PharMed tubing
   7.8. clamps
   7.9. aluminum foil
   7.10. air filters (4)
   7.11. glass wool
   7.12. probe stand with clamps
   7.13. 15mL conical tube
   7.14. O_2 electrolyte (for DO probe)
   7.15. 3M potassium chloride (KCL) solution for pH probe storage

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7.16. Bioreactor
- 7.16.1. glass pH probe
- 7.16.2. stainless steel DO probe
- 7.16.3. Pt-100 temperature probe
- 7.16.4. harvest tube
- 7.16.5. sparger
- 7.16.6. thermowell tube
- 7.16.7. glass vessel
- 7.16.8. condenser
- 7.16.9. feed bottle
- 7.16.10. sample bottle assembly
- 7.16.11. heater blanket

7.17. autoclave
7.18. biological safety cabinet
7.19. glycerol
7.20. transfer pipets
7.21. source of CO_2
7.22. source of air
7.23. 1L sterile bottles
7.24. 500mL sterile bottles

8. Procedure:

8.1. **Assemble Vessel Stand (Figure 1)**
- 8.1.1. Inspect the integrity of the large O-rings on the vessel stand and headplate.
- 8.1.2. Replace if worn or cracked.
- 8.1.3. Lower glass vessel into the vessel stand, making contact with the O-ring.
- 8.1.4. Verify that the bolts are threaded completely into the vessel stand. Thread if necessary.
- 8.1.5. If preparing for a run, add approximately 25mL 1X PBS to the vessel and approximately 250mL of 1M Sodium bicarbonate to the feed bottle.

8.2. **Assemble Headplate –Underside (Figure 2)**
- 8.2.1. Inspect the integrity of the O-rings on the harvest tube, sparger tube, and thermowell tube. Replace if worn or cracked.
- 8.2.2. Put two inch long piece of tubing on the non-barbed end of the harvest tube.
- 8.2.3. Insert the harvest tube from the underside of the headplate between the inoculation port and 3 addition port.
- 8.2.4. Place a washer and nut onto the harvest tube on the top of the headplate. Tighten the nut until the retainer of the harvest tube is flush with the underside of the headplate.
- 8.2.5. Insert the sparger tube from the underside of the headplate, next to blind stopper.
- 8.2.6. Rotate sparger tube so it is aligned beneath the stirrer impeller.

Title: Applikon Bioreactor Operation SOP

8.2.7. Place a washer and nut onto the sparger tube on the top of the headplate. Tighten the nut until the retainer of the sparger tube is flush with the underside of the headplate.
8.2.8. Insert the thermowell tube from the underside of the headplate, in between the pH and DO probe ports.
8.2.9. Place a washer and nut onto the thermowell tube on the top of the headplate. Tighten the nut until the retainer of the thermowell tube is flush with the underside of the headplate.

8.3. **Attach Headplate to Vessel Stand (Figure 3)**
8.3.1. Place the headplate onto the vessel stand, positioning the holes on the outer edge of the headplate with the bolts on the vessel stand.
8.3.2. Place the sample bottle assembly onto the bolt located by the 3 addition port and attach with a mill fastener.
8.3.3. Secure the headplate with the remaining 5 mill fasteners by hand in a crosswise pattern.

8.4. **Assemble Headplate – Top Side (Figure 3)**
8.4.1. Inspect the integrity of the O-ring in the condenser port of the headplate. Replace if worn or cracked.
8.4.2. Inspect the black seal at the bottom of the condenser underneath the retainer nut. Replace if worn or cracked.
8.4.3. Place the condenser into the condenser port making sure that the barbed connectors are facing out.
8.4.4. Tighten the retainer nut.
8.4.5. **DO Probe**
8.4.5.1. Remove the protective cap from the bottom of the stainless steel DO probe.
8.4.5.2. Inspect the screen at the bottom of the probe tip. Replace if damaged.
8.4.5.3. Holding the probe in a vertical position, unscrew the membrane module from the bottom of the probe.
8.4.5.4. Inspect the integrity of the O-ring underneath the module and replace if worn or cracked.
8.4.5.5. Replenish DO electrolyte. There should be approximately 1mL of 0_2 electrolyte solution in the membrane module.
8.4.5.6. Replace membrane module.
8.4.5.7. Inspect the integrity of the O-ring at the top of the stainless steel DO probe. Replace if worn or cracked.
8.4.5.8. Inspect the black seal at the top of the DO probe under the retainer nut. Replace if worn or cracked.
8.4.5.9. Place the DO probe into the DO probe port and tighten retainer nut.

8.5. **pH Probe Calibration**
8.5.1. Remove the protective caps from the top and bottom of the glass pH probe.
8.5.2. Pour the 3M potassium chloride (KCL) storage solution from the bottom protective cap into the 15mL conical tube.
8.5.3. Connect the pH Controller cable to the top of the pH probe.

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8.5.4. Turn on the ADI1025 unit by pressing the large green button on the front of the unit.
8.5.5. Wait for instrument to initialize (approximately one minute).
8.5.6. The set point main screen will appear on the display screen.
8.5.7. On the ADI1010, press the "pH" key then the "Calib." key.
8.5.8. Use the dial on the ADI1010 to highlight "reset to default calibration values."
8.5.9. Press the "Calib." key to input the factory defaults into the ADI1010 controller.
8.5.10. Press the "pH" key then the "Calib." key.
8.5.11. Use the dial on the ADI1010 to highlight the calibration option.
8.5.12. Press the "Calib." key to select the calibration option. This will display the pH calibration screen.
8.5.13. Rinse the pH and Pt-100 temperature probes with DI water and blot dry.
8.5.14. Place the pH and Pt-100 temperature probes into the pH 7 buffer standard.
8.5.15. Allow the pH and temperature readings to stabilize.
8.5.16. Press "Start/Stop" key to input the temperature value.
8.5.17. Press the "Calib." key.
8.5.18. Use the dial on the ADI1010 to input the pH value of the standard (7.00).
8.5.19. Press the "Calib." key.
8.5.20. Rinse the pH and Pt-100 probe with DI water and blot dry.
8.5.21. Place the pH and Pt-100 probe in to the pH 4 buffer standard.
8.5.22. Allow the pH reading to stabilize.
8.5.23. Use the dial on the ADI1010 to input the pH value of the standard (4.00).
8.5.24. Press the "Calib." Key. This will display the pH calibration slope and offset values.
8.5.24.1. Expected values:
Slope: 0.95-1.05
Offset: $< \pm 0.3$
8.5.24.2. See supervisor if not within this range.
8.5.25. Press the "Setp." key twice to return to the main screen.
8.5.26. Turn off power by pressing the large green button on the ADI1025 unit.
8.5.27. Remove both probes from pH 4 buffer standard.
8.5.28. Rinse both probes with DI water and blot dry.
8.5.29. Disconnect the pH probe from the controller cable and replace the top protective cap.
8.5.30. Inspect the integrity of the O-ring at the top of the glass pH probe. Replace if worn or cracked.
8.5.31. Inspect the black seal at the top of the pH probe under the retainer nut. Replace if worn or cracked.
8.5.32. Place the pH probe into the pH probe port and tighten the retainer nut.

Title: Applikon Bioreactor Operation SOP

8.6. **Attach Tubing/ Autoclave Preparation**

8.6.1. Connect labeled end of silicone tubing with air filters on the following :
sparger tube
condenser top outlet
CO_2 overlay port

8.6.2. Use a small piece of silicone tubing to connect together two of the ports on the 3 addition port.

8.6.3. Connect the PharMed tubing from the feed bottle to the 3 addition port.

8.6.4. Connect the sample bottle tubing to the harvest tube.

8.6.5. Clamp off all tubing (near the headplate) except the condenser top outlet. The condenser top outlet must remain unclamped to release pressure during autoclaving.

8.6.6. Close all open ends with glass wool and cover with aluminum foil.

8.7. **Autoclave**

8.7.1. Prepare autoclave per autoclave SOP.

8.7.2. Transfer bioreactor vessel to autoclave.

8.7.3. Autoclave at 121°C for 20 minutes, using slow exhaust.

8.7.4. Remove from autoclave when cycle is complete and return to bench.

8.8. **Polarize DO Probe**

8.8.1. Remove protective caps from the pH and DO probes.

8.8.2. Connect the pH and DO probes to the appropriate power cable.

8.8.3. Wrap the thermal blanket around the vessel and plug into the ADI1025.

8.8.4. Turn on power by pressing the large green button on the ADI1025 unit.

8.8.5. Allow DO probe to polarize for at least 6 hours before performing DO calibration.

8.9. **Media Addition**

8.9.1. Aseptically add media (per the process SOP) to the bioreactor vessel.

8.10. **Run Preparation**

8.10.1. Place stirrer motor on stirrer motor mount.

8.10.2. Remove the clamps on the following tubing:
feed bottle
CO_2 overlay port
sparger

8.10.3. Place tubing from the feed bottle through the feed pump on the ADI1025.

8.10.4. Connect the tubing from the CO_2 overlay port to the overlay outlet on the ADI1025.

8.10.5. Connect the tubing from the sparger to the sparger outlet on the ADI1025.

8.10.6. Using a transfer pipet, add a small amount (1.5-2mL) of glycerol to the thermowell.

8.10.7. Place the Pt-100 temperature probe into the thermowell.

8.11. **Establish Set Points**

8.11.1. On the ADI1010, press a parameter key (pH, Temp, dO_2, or Stirrer) then the "Setp." key.

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8.11.2. Use the dial to set the desired set point per the process SOP.
8.11.3. Press the "Setp." key to return to the Main Menu.
8.11.4. Repeat until each set point in the process SOP is established per the process SOP.

8.12. **Establish Limits**
8.12.1. On the ADI1010, press a parameter key.
8.12.2. Press the "limits" key to go to the upper limit.
8.12.3. Use the dial to set the upper limit per the process SOP.
8.12.4. Press the 'limits" key again to go to the lower limit.
8.12.5. Use the dial to set the lower limit per the process SOP.
8.12.6. Press the "Setp." key to return to the Main Menu
8.12.7. Repeat until an upper and lower limit is set for each parameter per the process SOP.

8.13. **Activate Control Loops**
8.13.1. Press the pH key then the "Start/Stop" key to activate the pH control loop.
8.13.2. Press the "Temp" key then the "Start/Stop" key to activate the temperature control loop.
8.13.3. Press the "Stirrer" key then the "Start/Stop" key to activate the stirrer control loop.
8.13.4. Lights on the pH, temperature, and stirrer keys should turn green.
8.13.5. NOTE: Do NOT activate the dO_2 control loop at this time.

8.14. **DO Probe Calibration (after six hours of polarization)**
8.14.1. On the ADI1010, press the "dO_2" key then the "Calib." key.
8.14.2. Four options are displayed: (View Calibration Values, Temperature Compensation, Calibration, and Reset to Default Calibration Values.)
8.14.3. Use the dial on the ADI1010 to highlight the calibration option.
8.14.4. Press the "Calib." key to select the calibration option.
8.14.5. Use the dial on the ADI1010 to set the value to100.
8.14.6. Press the "Calib." key.
8.14.7. The DO probe calibration slope should be on the ADI1010 display.
8.14.7.1. Expected values are:
Slope: 8-15 at 37^{o}C or 10-20 at 25^{o}C
8.14.7.2. See supervisor if not within this range.
8.14.8. Press the "Setp." key to return to the main screen on the ADI1010 display.
8.14.9. Press the "dO_2" key then the "Start/Stop" key to activate the dO_2 control loop.
8.14.10. Lights on the "dO_2" key should turn green.
8.14.11. Disconnect the DO probe BRIEFLY (<1min) from the DO cable.
8.14.11.1. The DO reading should quickly decrease to 0.
8.14.11.2. If the DO value does not drop below 0.5% the probe requires maintenance (refer to the user manual for the DO probe).
8.14.11.3. Reconnect the DO cable to the DO probe.
8.14.12. Turn on Air and CO_2 supplies to the bioreactor.

Title: Applikon Bioreactor Operation SOP

8.15. **Add cell culture through the Inoculation port per the process SOP.**

8.16. **BioXpert Lite**

- 8.16.1. Turn on power to computer.
- 8.16.2. Open BioXpert Lite software.
- 8.16.3. Choose: Username: NHCTC, Mode: Cultivation, and Fermentor no.:1.
- 8.16.4. Select "OK".
- 8.16.5. To ensure the computer is communicating with the controller, on the menu bar, click on Run, Test. Next to each variable, a number should be displayed in the values column representing the value of that parameter.
  - 8.16.5.1. If no numbers are displayed, notify your instructor.
- 8.16.6. Select "OK".
- 8.16.7. On the menu bar, click on Run, New.
- 8.16.8. In the Organism box, type in the name of the cells being grown. Type any comments in the Comments box.
- 8.16.9. Select "OK".
- 8.16.10. Choose the access interval between readings.
  - 8.16.10.1. This can be changed during a run without changing the start moment.
- 8.16.11. Under Protocol in the Data box type "F" (for fall) or "S" (for spring) and the year (i.e. F2006).
  - 8.16.11.1. Note: The Data box allows a maximum of 8 characters.
- 8.16.12. Select "OK".
  - 8.16.12.1. Note: Selecting "OK" begins the data collecting process.
- 8.16.13. **Important:** Do NOT close (i.e. "X-out of) the On-line Session window or select "END" at **any** time during the run. Doing this will cause the **loss** of **all** data for the run.
- 8.16.14. To view the Chart window during a run, simply click on the blue bar at the top of the Chart window. This will bring the chart to the front. Do the same to view the On-line Session window if it is behind the Chart window.

8.17. **Setting the scale of the graph**

- 8.17.1. View the Chart window in the BioXpert light application by clicking on the blue bar at the top of the chart window.
- 8.17.2. View the "Y" axis variables by clicking on a parameter in the Variables window. All of the variables can be viewed at once.
- 8.17.3. Use the mouse to hold the curser over any of the Y axis variable lines (pH, Temp, dO_2, or Stirrer) in the chart. Left click to open the Scale Settings window.
- 8.17.4. Check the box next to "Manually Scale".
- 8.17.5. Choose the Y axis you would like to change in the "List of Variables" pull-down menu.
- 8.17.6. Change the scale by entering the desired values in the Scale range boxes.
- 8.17.7. Select "OK".

8.18. **Ending a Run**

- 8.18.1. On the menu bar, click on File, Save as. Delete the (*) and enter a file name.

Title: Applikon Bioreactor Operation SOP

8.18.2. Select "OK".
8.18.3. Export the data to EXCEL by choosing File, Export, Data on the menu bar.
8.18.3.1. Select the following options for export:
Format: SYLK (for EXCEL)
Variables: On-lines (Off-lines should not be checked.)
Time Interval: On-line
Time Range: All
8.18.3.2. Note: In the Time Interval option, On-line will export all data collected at the interval period chosen in section 8.16.10 while Off-line will export data points that were collected every 15 minutes. "Other" is used to export a custom interval**.**
8.18.3.3. Select "OK".
8.18.3.4. Under File Name delete the (*) and type a file name. Make note of the directory where the file is saved before selecting "OK".
8.18.3.5. Select "OK".
8.18.4. Turn off the control loops on the ADI1010by selecting a parameter key ("pH", "Temp", "dO_2", or "Stirrer"), and pressing the "Start /Stop" key. Repeat for each control parameter.
8.18.5. Turn off the Air and CO_2 supplies to the ADI1025 controller.
8.18.6. Aseptically remove cultivation through the harvest port, utilizing the sample bottle.
8.18.7. Turn off power switch on the ADI1025.
8.18.8. Remove stirrer motor.
8.18.9. Unplug the heater blanket and remove from the vessel. Store flat.
8.18.10. Disconnect the power cords for the pH and DO probes.
8.18.11. Remove the pH and DO probes and place in storage clamps.
8.18.12. Place protective caps on the pH and DO probes.
8.18.13. Remove the Pt-100 probe, rinse with DI water and blot dry.
8.18.14. Remove all tubing.

8.19. **Cleaning the Bioreactor**
8.19.1. Clean pH and DO probes.
8.19.1.1. Remove pH and DO probes from the headplate.
8.19.1.2. Wipe down DO and pH probes with a lab towel damp with 10% bleach solution.
8.19.1.3. Rinse DO and pH probes with DI water and blot dry.
8.19.2. Remove the headplate from the vessel (with harvest tube, thermowell tube and sparger tube still attached.
8.19.3. Add 1L of 10% bleach solution to the vessel.
8.19.4. Replace the headplate and soak the sparger tube, harvest tube and thermowell tube for approximately 15 minutes.
8.19.5. Remove headplate from vessel.

Title: Applikon Bioreactor Operation SOP

8.19.6. Disassemble the entire headplate assembly and wash all of the components with 10% bleach solution. Rinse with DI water, and spray with 70% IPA. Set out on lab towels to air dry.
8.19.7. Clean all tubing with 10% bleach solution, rinse with DI water, and spray with 70% IPA. Set out on lab towels to air dry.
8.19.8. Wipe down controller with a lab towel damp with a 10% bleach solution, then with a towel damp with DI water.
8.19.9. Place headplate and components out on lab towels to dry.
8.19.10. After the components have dried, reassemble the bioreactor for storage (leaving out the pH and DO probes).

8.20. **pH probe Storage**
8.20.1. After cleaning the pH probe, verify that no broth or media residue remains on the membrane surface or diaphragm.
8.20.2. Pour the 3M potassium chloride (KCl) solution from the 15mL conical tube into the protective cap. Add more 3M potassium chloride (KCl) solution if necessary to fill the cap ½ full with the solution.
8.20.3. Insert the electrode end of the pH probe into the protective cap. The electrode should be completely immersed in the 3M potassium chloride (KCl).
8.20.4. Place the probe in a storage clamp and store in vertical position.

8.21. **DO probe Storage**
8.21.1. After cleaning the DO probe, verify that no broth or media residue remains on the membrane surface or diaphragm.
8.21.2. Holding the probe in a vertical position, unscrew the membrane module from the bottom of the probe and verify that approximately 1mL of O_2 electrolyte solution is present in the membrane module.
8.21.3. Replace the protective cap. Place probe in storage clamp, and store in vertical position.

9. Attachments:
9.1. Figure 1: Vessel and Headplate
9.2. Figure 2: Underside of the Headplate
9.3. Figure 3: Assembled Headplate
9.4. Figure 4: ADI1010 Controller

10. History:

Name	Date	Amendment
Bob O'Brien Deb Audino	072705	Initial Release
Deb Audino Bob O'Brien	100605	Deb Audino and Bob O'Brien, 100605, Removed all grease steps, reduced amount of PBS to add to vessel, removed PBS removal step, modified adding media in BSC, modified cleaning steps.
Bob O'Brien	042706	Add O_2 in precaution, additional materials for probe storage, modify section 8.5 for calibration. Add section 8.20 pH probe storage; add section 8.21 DO probe storage.
Kari Britt	09Oct06	New directions for BioXpert Lite section to correspond with

Title: Applikon Bioreactor Operation SOP

		upgrade to new version to computers. Moved Export the Data section to the Ending a run section in order to consolidate BioXpert Lite directions.
Bob O'Brien	04Apr08	College name change

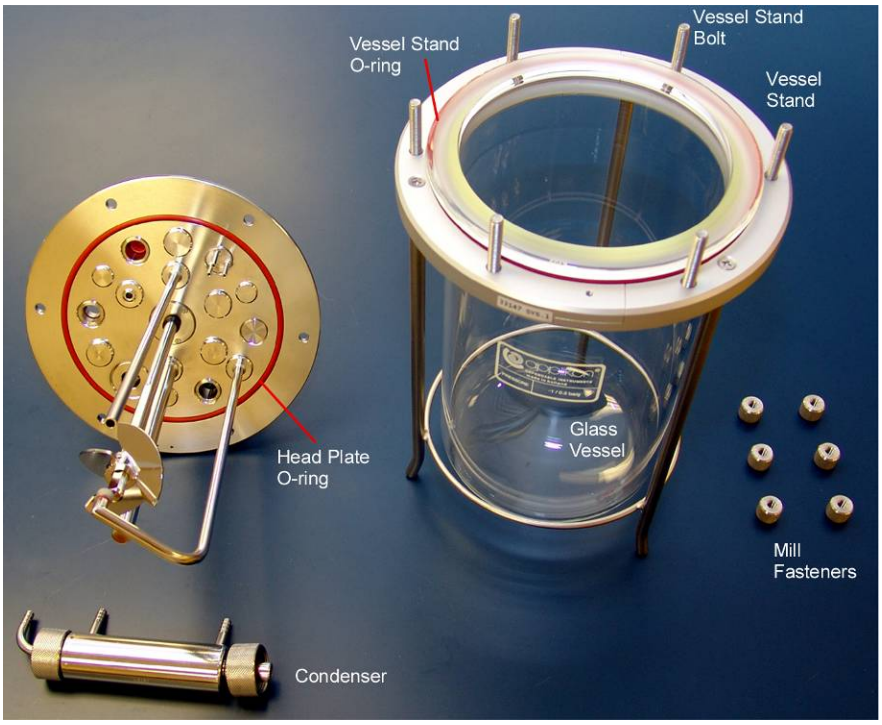

Figure 1: Vessel and Headplate

Title: Applikon Bioreactor Operation SOP

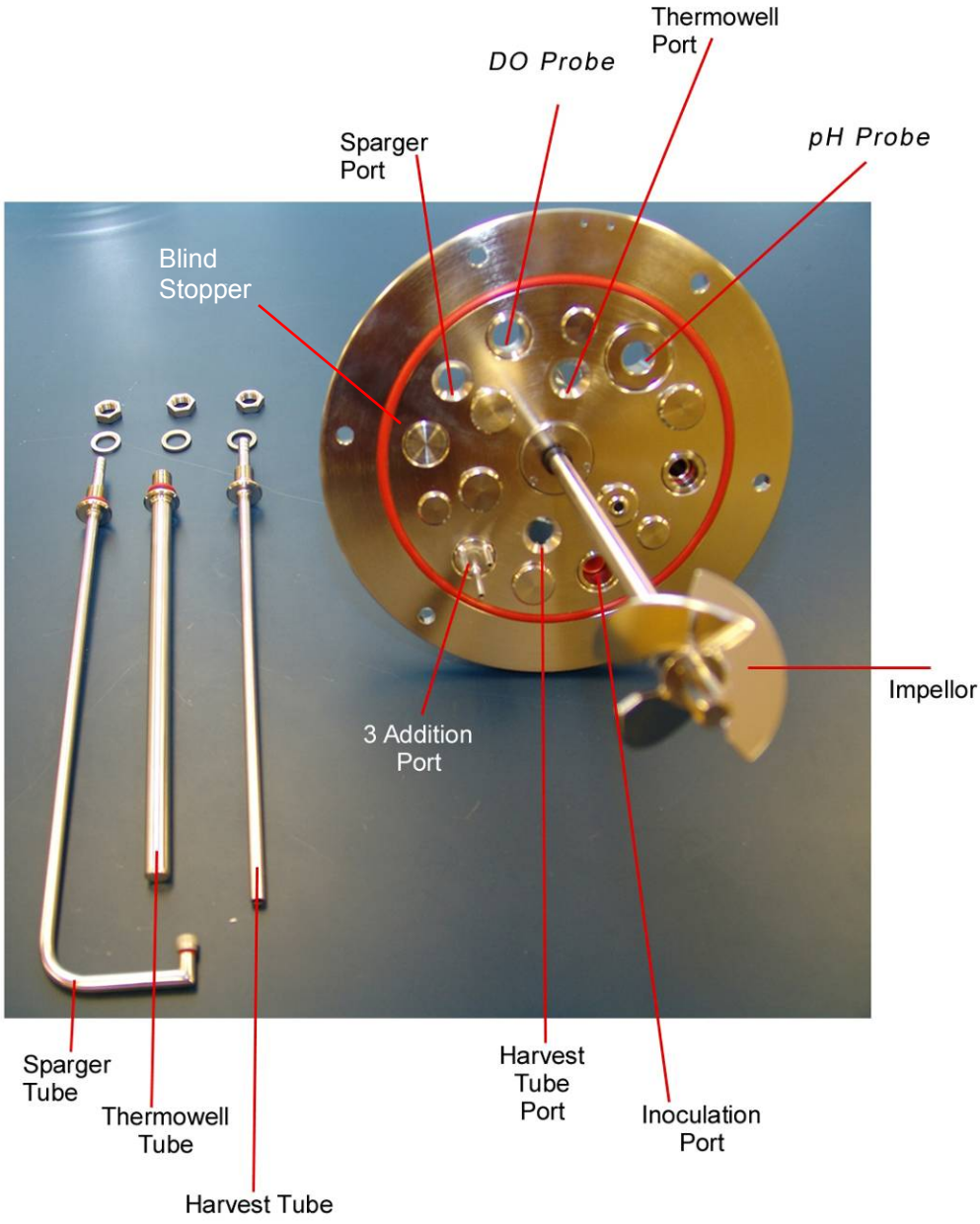

Figure 2: Underside of Headplate

Title: Applikon Bioreactor Operation SOP

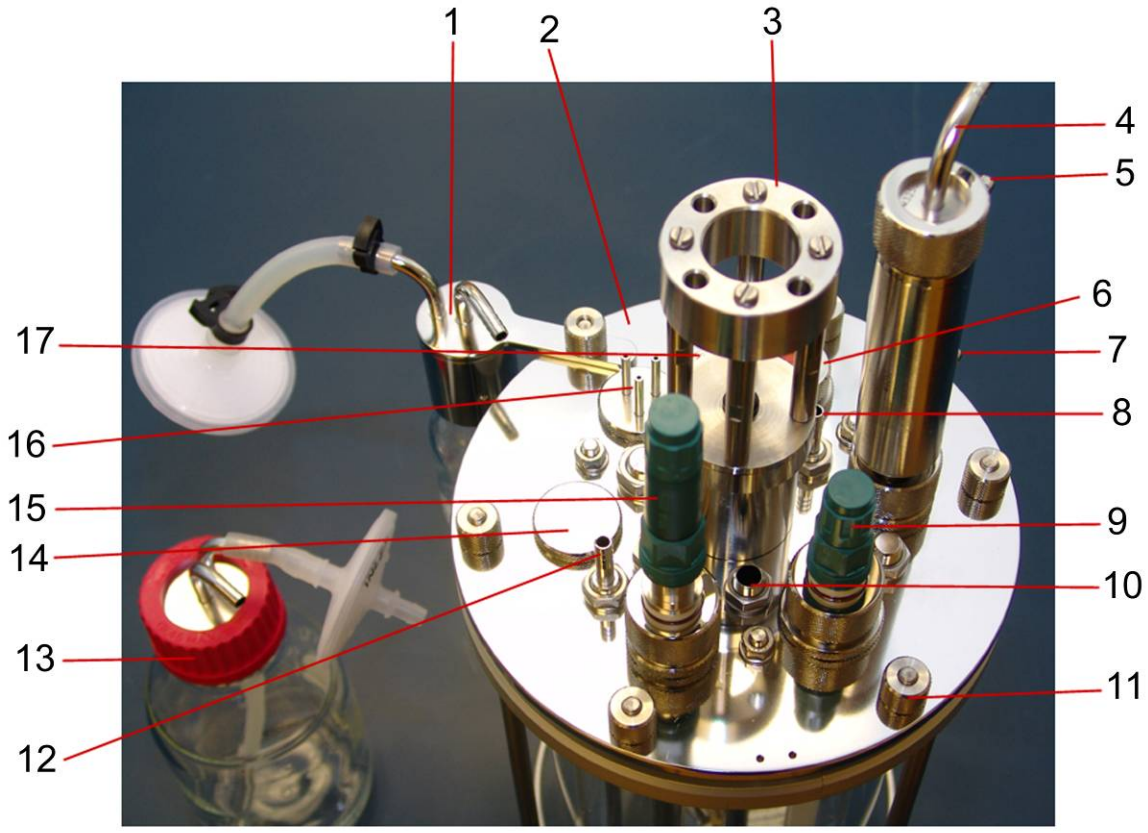

Figure 3: Assembled Headplate

1. Sample bottle assembly
2. Head plate assembly
3. Stirrer motor mount
4. Condenser air outlet
5. Condenser water outlet (from)
6. Inoculation port
7. Condenser water inlet (to)
8. CO_2 overlay port
9. pH probe
10. Thermowell port
11. Mill fastener
12. Sparger
13. Feed bottle
14. Blind stopper
15. DO probe
16. 3 Addition port
17. Harvest tube

Title: Applikon Bioreactor Operation SOP

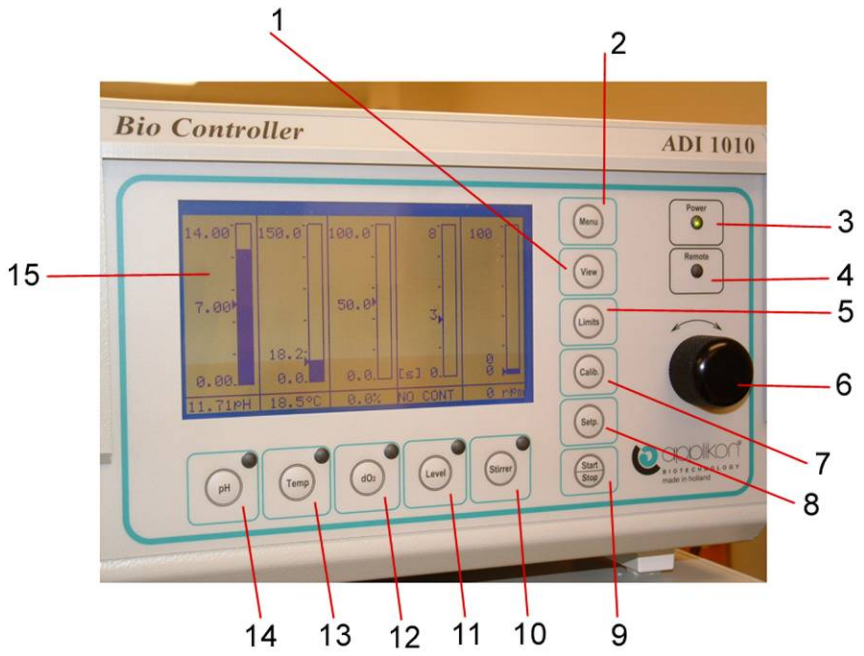

Figure 4: ADI1010 Controller

1. View function key.
2. Menu function key
3. Power indicator LED
4. Remote indicator LED
5. Limits function key
6. Dial: digital potentiometer
7. Calib. Key
8. Setup key
9. Start/Stop key
10. Stirrer parameter key; has a dual-color LED, off, Green on no alarm, Red out of range, and alternating Green/Red out of range
11. Level parameter key; has a dual color LED, off, Green on no alarm, Red out of range, and alternating Green/Red out of range
12. DO parameter key; has a dual-color LED, off, Green on no alarm, Red out of range, alternating Green/Red out of range
13. Temp parameter key; has a dual-color LED, off, Green on no alarm, Red out of range, alternating Green/Red out of range
14. pH parameter key; has a dual-color LED, off, Green on no alarm, Red out of range, alternating Green/Red out of range
15. Display screen

Title: Trypan Blue Assay SOP

Approvals:
Preparer:_____Kari Britt _____ Date _____05Aug10_____
Reviewer:_____Sonia Wallman_____ Date _____05Aug10_____

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.

Title: Trypan Blue Assay SOP

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. Left button is for live cells. Right button is for dead cells. Make sure the counter is set to 0.

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.

8.5. **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 deionized (DI) water 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 counted in the four main quadrants
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

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

Title: Trypan Blue Assay SOP

10. History:

Name	Date	Amendment
Kari Britt	26Jul05	Initial release
Deb Audino	04Apr08	College name change, format of history
Kari Britt	05Aug10	Proofreading, formatting and grammar edits throughout

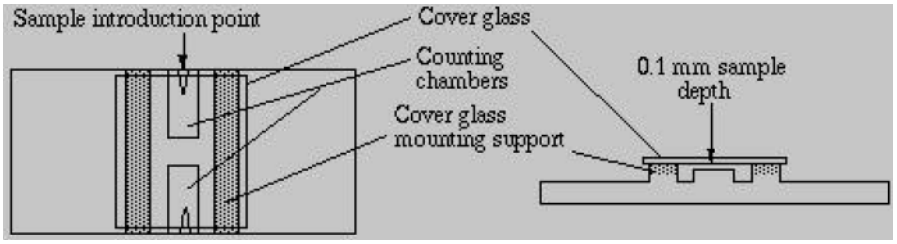

Figure 1: Diagram of hemacytometer and cover glass
Image: http://plaza.ufl.edu/johnaris/Protocols/MiscMethods/HemoCytometer.pdf

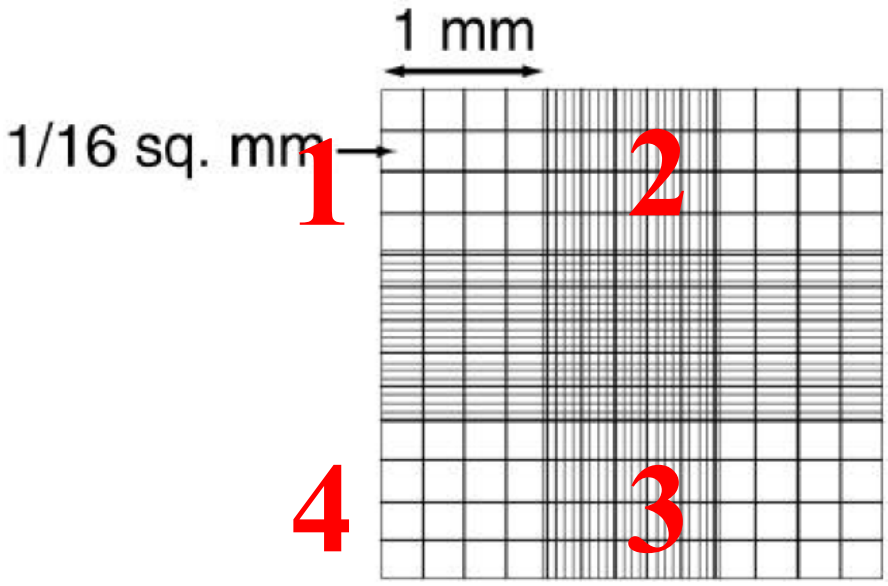

Figure 2: Diagram of hemacytometer quadrants

Kodak IBI Biolyzer Operation SOP

Approvals:
Preparer:_____ Deb Audino _____ Date _____03Apr08_____
Reviewer:_____Bob O"Brien_____ Date _____03Apr08_____

1. **Purpose:**
   1.1. Operation of the Kodak IBI Biolyzer Rapid Analysis.
2. **Scope:**
   2.1. Applies to the operation of the Kodak IBI Biolyzer Rapid Analysis System to track vital analyte consumption/production of living organisms.
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. Kodak IBI Biolyzer Manual.
   4.2. Kodak EKTACHEM DT pipette SOP.
5. **Definitions:**
   5.1. Analyte: chemical/substance being measured.
6. **Precautions:**
   6.1. Do not open spotting locator cover or the unit will have to reboot and warm-up again.
7. **Materials:**
   7.1. Vitros slides from the -20°C freezer.
   7.2. Kodak EKTACHEM DT pipette
   7.3. Kodak EKTACHEM DT pipette tips.
   7.4. charge cord for pipette.
8. **Procedure:**
   8.1. Instrument Preparation
      8.1.1.Turn on the instrument. Allow the instrument to warm up for 15-20 minutes prior to using.
      8.1.2.Plug in the charge cord to the Kodak pipettor. Make sure red plug shows in the window. If not, then depress the black-square button until it does.
      8.1.3.Remove the test slides from the freezer and/or refrigerator. Allow them to reach room temperature before using (15-30 minutes).
   8.2. Maintenance
      8.2.1.Perform daily maintenance prior to using the instrument by emptying the slide disposal box on the right rear of the unit and checking the paper level.
   8.3. Sample testing
      8.3.1.Enter the date in the Biolyzer by the following steps:
         8.3.1.1.Press the SHIFT then the SERVICE key.
         8.3.1.2.At the OPTION prompt, type "17" then ENTER.

Kodak IBI Biolyzer Operation SOP

8.3.1.3. Enter date as MM-DD-YY, then ENTER. NOTE: this will return you to the OPTION prompt.

8.3.1.4. Exit out of service mode by pressing the SHIFT then the SERVICE key.

8.3.2. Remove the slide to be tested from the foil package. NOTE: slides must be used within 15 minutes of opening a package.

8.3.3. Place the slide on the slide track, line up slide as shown on slide lever. Manually push the slide advance lever to move the slide into the spotting position. NOTE: console should read: "spot slide with fluid."

8.3.4. Enter ID of specimen (if necessary) by pressing SAMPLE ID key and enter sample name/number. If no ID is necessary the machine will automatically assign it a numerical number (ie. 1,2,3, etc.)

8.3.5. Using the Kodak EKTACHEM DT pipette, remove 10 µl of the test sample. Refer to the Kodak EKTACHEM DT pipette SOP for proper use.

8.3.6. Insert the pipette tip into the spotting locator (hole above slide) ensuring pipette is properly seated and dispense the sample. NOTE: The console should read: "Wait- slide being loaded."

8.3.7. Wait for the console to read: "Ready." Remove the pipettor from the locator and discard the pipette tip. Repeat steps 8.3.2 – 8.3.6 for each analyte to be tested. NOTE: slides will advance into slide disposal box.

8.4. Interpretation

8.4.1. The values will print shortly after procedure is completed

9. Attachments: N/A

10. History:

Name	Date	Amendment
Sonia Wallman	1997	Initial release
Zach Bodah	18Feb05	Updated to 2005 SOP format
Deb Audino	04 Apr08	College name change

Kodak EKTACHEM DT Pipettor SOP

Approvals:
Preparer:_____ Deb Audino_____ Date _____03Apr08_____
Reviewer:_____Bob O'Brien_____ Date _____03Apr08_____

1. **Purpose:**
   1.1. Operation of the Kodak EKTACHEM DT Pipettor.
2. **Scope:**
   2.1. Applies to the Kodak EKTACHEM DT Pipettor used for accurately dispensing sample fluids into the Kodak IBI Biolyzer spotting locator.
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. IBI Biolyzer Manual V. 3.0.
   4.2. Kodak IBI Biolyzer SOP
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. Kodak EKTACHEM DT pipette tips.
   7.2. charge cord for pipette.
   7.3. Kodak IBI Biolyzer.
8. **Procedure:**
   8.1. Check Pipette
      8.1.1.Plug charge cord into the pipette and power ON.
      8.1.2.Check the pipette read window. The red indicator dot shows that the pipette is ready for operation. If the red dot is not showing, depress the button and release once.
   8.2. Insert Tip
      8.2.1.To attach a disposable tip, press the pipette firmly into one of the tips in the holder. The tip will click into place when it is seated.
   8.3. Aspirate fluid
      8.3.1.Hold pipette in vertical position.
      8.3.2.Insert tip into fluid, but not to the bottom of the fluid container.
      8.3.3.Depress button and release it (10µL of fluid is automatically drawn into pipette). Note: Do not depress the button more than once.
      8.3.4.A tone will be heard, which is the signal to withdraw the pipette. Remove the pipette from the fluid immediately.
      8.3.5.A second tone will be heard. This indicates that the pipette is stll drawing the fluid further into the tip, to prevent accidental loss of fluid.

Kodak EKTACHEM DT Pipettor SOP

8.3.6. Hold the pipette vertically whenever there is fluid in the tip. If you tilt the pipette or lay it on its side, fluid might enter the mechanism causing it to become clogged. If this occurs, clean the pipette immediately.

8.4. Remove excess fluid

8.4.1. To remove any droplets which may be clinging to the outside of the tip, take a laboratory tissue and wipe the outside of the tip in a light, quick motion. If the tip is not wiped, test results may be inaccurate. Visually check the fluid level.

8.5. Spot the slide with fluid

8.5.1. Depress and release the dispenser button. The Biolyzer console should read: "WAIT-slide being loaded." Wait for the console to read: READY before removing from the spotter locator.

8.6. Check pipette tip

8.6.1. Check that the fluid was completely dispensed from the tip.

8.6.2. Eject tip by pressing the eject button on the pipettor.

9. Attachments: N/A

10. History:

Name	Date	Amendment
Zach Bodah	18Feb05	Initial release
Deb Audino	04Apr08	College name change

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. Media Preparation		
Clean, assemble and autoclave two 100mL Bellco Spinner flasks per SOP. Spinner flask ID#_____Spinner flaskID#_____	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 90mL ± 1 mL 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 10mL ± 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 each of the previously prepared Spinner Flask containing 100mL CHO Cell Culture Medium using a 2mL sterile pipette. **Swirl** to mix.	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_____

3. 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_____

4. Assemble/Autoclave Bioreactor		
4.1. 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
4.2. 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
4.3. 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
4.4. 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
4.5. Attach Filters and Tubing		
Place silicone tubing on the Sparger tube,Condenser top outlet, and CO_2 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_____

5. Media Preparation and Addition / Run Preparation		
Place the bioreactor and a sterile funnel in the Biological Safety Cabinet and expose to UV light for 20-30 minutes.	Operator/Date	Verifier/Date
Add approximately 6mL of 200mM Glutamine and 10mL of 10mg/mL gentamycin to a 1L bottle of ProCHO4 media. Pour into bioreactor. ProCHO4 media: Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Glutamine: 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 glycerol 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** **Lower limit** pH 7.3 7.1 Temperature 38 36 DO 52 48 Temperature 38 36 Agitation 76 74	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

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: 8-15 at 37°C or 10-20 at 25°C	Operator/Date	Verifier/Date
Turn on Air supply at regulator 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
Turn on computer and open BioXpert Lite software per Applikon Bioreactor Operation SOP. **Name** the file. File Name: _____	Operator/Date	Verifier/Date
Ensure the computer is communicating with the controller per the Applikon Bioreactor Operation SOP. **Click** the OK button to begin the data collection process. **IMPORTANT** – In the On-Line Session window **DO NOT CLICK ON *END***. This will end the on-line session and stop collecting data.	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_____

6. Ending a Run		
Save the file for the run. File Name: _____	Operator/Date	Verifier/Date
Turn off each control loop. Turn off the supply of Air the ADI1025 controller. Turn off the supply of CO_2 supplied to the ADI1025 controller.	Operator/Date	Verifier/Date
Aseptically remove the culture through the harvest port.	Operator/Date	Verifier/Date
Clean the pH , DO, and the Pt-100 probes with a 10% bleach solution, and rinse with DI water. Place protective caps on the pH probe. Place protective caps on the DO probes.	Operator/Date	Verifier/Date
Clean the bioreactor.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date
7. 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_____

8. Prepare storage menstrum:		
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 Menstrum 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 menstrum 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_____

9. Prepare Growth Curves		
Plot OD, pH, viable cells, glucose, and lactatevs. time (use 2 y-axes). Attach graph to Batch Record.	Operator/Date	Verifier/Date
Determine growth rate and doubling time of the 50mL and 500mL cultures (Show calculation) Growth Rate 100mL ID#_____is_____ Growth Rate 100mL ID#_____is _____ Growth Rate bioreactor ID#_____is _____	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

Manufacturing Technician (Upstream)

REF	Key Functions & Tasks (Upstream Manufacturing Technician)
1	**Work in compliance with EH&S.**
1.a	Wear appropriate personal protective equipment.
1.b	Work in controlled environments.
1.c	Participate in emergency drills and emergency response teams.
1.d	Identify unsafe conditions and take corrective action.
1.e	Appropriately and safely access production equipment.
1.f	Handle, label, and dispose of hazardous / biohazard materials.
1.g	Access and utilize MSDS.
1.h	Perform permitting procedures.
1.i	Carries out operations with attention to OSHA and EPA regulations, and other applicable state and federal regulations.
1.j	Keeps work areas clean and safety equipment in order.
1.k	Participate in all company safety training and audits as required.
1.l	Assists with waste treatment operations.
2	**Work in compliance with cGMPs.**
2.a	Assists in environmental monitoring activities.
2.b	Follow SOPs for all operations.
2.c	Records process data and completes batch records as required.
2.d	Maintain equipment logbooks.
2.e	Control and receipt of raw materials.
2.f	Maintain training documentation.
2.g	Maintain equipment and processes in a validated state.
2.h	Working in controlled/classified areas (gowning, aseptic technique).
2.i	Ensure appropriate flow of personnel, equipment, and materials.
2.j	Change control for process, equipment, and documentation.
2.k	Label and apply status to equipment and materials.
2.l	Identify and report exception events and CAPA.
3	**Clean and maintain production areas.**
3.a	Housekeeping / pest control.
3.b	Sanitize and clean of controlled spaces.
3.c	Preparation of cleaning materials and solutions.
3.d	Assist in environmental monitoring for routine and changeover operations.
3.e	Document cleaning.
4	**Maintain effective communication.**
4.a	Deliver shift change update.
4.b	Communicate with coworkers and/or customers to ensure production or service meets requirements.
4.c	Suggest continuous improvements.
4.d	Coordinate with work teams / internal customers.
4.e	Maintain security and confidentiality.
4.f	Respond appropriately to internal auditors and external inspectors.
4.g	Assist in writing, reviewing, and commenting on technical documents.

REF	Key Functions & Tasks (Upstream Manufacturing Technician)
5	**Prepare process materials.**
5.a	Weigh, dispense, and label raw materials for use in production.
5.b	Dispense consumables and intermediates.
5.c	Control and reconcile inventory with enterprise control system (MRP, SAP, manual database).
5.d	Prepare and sterilize buffers and solutions.
5.e	Sample and test buffers and solutions.
5.f	Transfer buffers and solutions to use point.
5.g	Prepare filters for use.
5.h	Prepare, pasteurize / sterilize, and titrate media and feed solutions.
5.i	Manage chromatography resins.
6	**Prepare equipment.**
6.a	Clean CIP vessels, transfer lines, and filter trains .
6.b	Clean COP equipment (or sonicator).
6.c	Depyrogenate components and equipment.
6.d	Sterilize SIP vessels, transfer lines, and sampling ports.
6.e	Perform pressure test.
6.f	Prepare and assemble components and equipment.
6.g	Autoclave components and equipment.
6.h	Perform WFI flush of transfer lines.
6.i	Perform scheduled sanitizations of hoods.
6.j	Prepare and standardize probes and ancillary instruments.
6.k	Prepare, assembly, and integrity test filters.
6.l	Visually inspect equipment.
6.m	Maintain equipment logs and status tags.
6.n	Complete, review and approve equipment process records.
7	**Perform basic manufacturing operations.**
7.a	Perform processes following batch records, validation protocols, and/or SOPs.
7.b	Maintains and controls processes in an automated control environment.
7.c	Record process data.
7.d	Inspect materials at all stages of process to determine quality or condition.
7.e	Participate in the installation, modification, and upgrade of equipment.
7.f	Operate, monitor, and maintain equipment, tools, and workstation.
7.g	Recognize and respond appropriately to atypical events.
7.h	Participate in troubleshooting and root cause analysis of operations.
8	**Perform upstream manufacturing operations.**
8.a	Work in an aseptic environment (laminar flow hood / biosafety cabinet / cleanrooms).
8.b	Perform vial thaw from a working cell bank.
8.c	Perform cell culture expansion.
8.d	Monitor cell concentration by cell counting or measuring OD.
8.e	Inoculate seed reactor.
8.f	Transfer of seed culture to production reactors.
8.g	Monitor and control growth of cells in batch, fed-batch, and perfusion reactors.
8.h	Perform aseptic additions of media, solutions, and/or gases to reactors.
8.i	Perform CIP/SIP of bioreactors.
9	**Perform Sampling.**
9.a	Prepare sample port for aseptic sampling.
9.b	Obtain in-process samples according to batch records or sampling plans.
9.c	Label samples appropriately.
9.d	Record sample collection and distribution (storage and chain of custody).
9.e	Perform in-process chemical and/or microbiological tests.

Title: Ion Exchange Chromatography of tPA SOP

Approvals:
Preparer:_____Kari Britt_____ Date ____02Jun09_____
Reviewer:_____Bob O'Brien_____ Date ____02Jun09_____

1. **Purpose:**
   1.1. To purify tPA using ion exchange chromatography.
2. **Scope:**
   2.1. Applies to purifying tPA using POROS 50HS resin and the BioLogic LP system.
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. POROS 50HS Manufacturer's Instructions.
   4.2. pH meter SOP
   4.3. Amicon/Millipore column assembly SOP
   4.4. BioLogic LP SOP
5. **Definitions:**
   5.1. CV: Column Volume; CV = π(L in cm)[(radius of column in cm)^2]
   5.2. L = Length of column (meaning the height of the bead bed)
   5.3. HETP: Height Equivalent to Theoretical Plate; HETP = L/N
   5.4. $N = 5.54\ (t_R/w_{1/2})^2$
   5.5. t_R: retention time
   5.6. $w_{1/2}$: peak width at half height
   5.7. h: Reduced Plate Height; h = HETP/Dp
   5.8. Dp: bead diameter
6. **Precautions:**
   6.1. 0.1M NaOH is very corrosive. It is extremely damaging to eyes and mucous membranes. It causes burns. Avoid contact with skin. Harmful if swallowed or inhaled.
7. **Materials:**
   7.1. Amicon Vantage-L Biochromatography column and accessories
   7.2. POROS 50 HS Cation exchange packing medium (2-8°C)
   7.3. BioRad BioLogic LP System
   7.4. 0.22 µm sterile filter units
   7.5. pH paper
   7.6. waste beakers
   7.7. laboratory film, such as, Parafilm
   7.8. ring stand with clamps
   7.9. 1mL syringe
   7.10. MilliQ water
   7.11. Equilibration Buffer A: 20mM phosphate buffer pH 6
      7.11.1. NaH_2PO_4 (sodium phosphate monobasic, anhydrous)

Title: Ion Exchange Chromatography of tPA SOP

7.11.2. Na_2HPO_4-$7H_2O$ (sodium phosphate dibasic, heptahydrate)
7.12. Elution Buffer B: 20mM phosphate buffer pH6, 1M NaCl
7.12.1. NaH_2PO_4 (sodium phosphate monobasic, anhydrous)
7.12.2. Na_2HPO_4-$7H_2O$ (sodium phosphate dibasic, heptahydrate)
7.12.3. NaCl (sodium chloride)
7.13. Cleaning Solution 0.1M NaOH

8. Procedure:

8.1. **Prepare buffers and solutions**

8.1.1. **Buffer A: Equilibration Buffer, 20mM Phosphate, pH 6**

8.1.1.1. Weigh 2.10 ±0.05 grams of sodium phosphate monobasic anhydrous.
8.1.1.2. Weigh 0.66 ±0.02 grams of sodium phosphate dibasic heptahydrate.
8.1.1.3. Transfer both chemicals to a 1L vessel.
8.1.1.4. Using a 1L graduated cylinder, measure approximately 1L of deionized water.
8.1.1.5. Transfer water to the 1L vessel.
8.1.1.6. Add magnetic stir bar and stir to dissolve.
8.1.1.7. Adjust pH to 6 ±0.1.
8.1.1.8. Filter sterilize.
8.1.1.9. Label as: Buffer A, Equilibration Buffer, 20mM Phosphate, pH 6, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].

8.1.2. **Buffer B: Elution Buffer, 20mM phosphate, pH 6, 1M NaCl**

8.1.2.1. Weigh 29.2 ±0.2 grams NaCl.
8.1.2.2. Transfer to a 1L vessel.
8.1.2.3. Using a 500mL graduated cylinder, measure approximately 500mL of 20mM phosphate buffer pH 6.
8.1.2.4. Transfer to the 1L vessel with the NaCl.
8.1.2.5. Add magnetic stir bar and stir to dissolve.
8.1.2.6. Filter sterilize.
8.1.2.7. Label as: Buffer B, Elution Buffer, 20mM Phosphate, pH 6, 1M NaCl, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].

8.1.3. **Cleaning Solution: 0.1M NaOH**

8.1.3.1. Weigh 2.0 ±0.05 grams of NaOH.
8.1.3.2. Transfer NaOH to a 1L vessel.
8.1.3.3. Using a 500mL graduated cylinder, measure 500mL of deionized water.
8.1.3.4. Transfer to the 1L vessel.
8.1.3.5. Add magnetic stir bar and stir to dissolve.
8.1.3.6. Filter sterilize.
8.1.3.7. Label as: Cleaning Solution, 0.1M NaOH, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].

8.1.4. **Buffer C: CHO Cell Culture Supernatant, pH 6**

8.1.4.1. Adjust the pH of the CHO cell culture supernatant obtained during upstream processing of tPA to pH 6.
8.1.4.1.1. Measure the pH of the CHO cell culture supernatant per pH meter SOP.

Title: Ion Exchange Chromatography of tPA SOP

8.1.4.1.2. If the initial pH is above 6 ±0.1, carefully add one drop of hydrochloric acid solution to the supernatant.
8.1.4.1.3. Observe the change in pH.
8.1.4.1.4. Repeat step 8.1.4.1. until the supernatant solution is pH 6 ±0.1.
8.1.4.2. Label as: Buffer C, CHO Cell Culture Supernatant, pH 6, Store: 2-8°C, Dispose: drain, [date], [group], [initials].

8.2. **Purge BioLogic LP Sytem with Buffer A and zero the UV monitor per the Biologic LP Chromatography System SOP**

8.3. **Pour Column per the BioLogic LP Chromatography System SOP**

8.3.1. Use approximately 5mL of POROS HS resin.

8.4. **Attach the column to the BioLogic LP per the BioLogic LP Chromatography System SOP**

8.5. **Pack Column per the BioLogic LP Chromatography System SOP**

8.5.1. Place the line for Buffer A into the vessel containing Buffer A, Equilibration Buffer. Cover the opening of the vessel with laboratory film, such as Parafilm.

8.5.2. Use Method: IEX Pack:

Step 1:	0-5min	Buffer A	1.0mL/min
Step 2:	5-10min	Buffer A	2.0mL/min
Step 3:	10-20min	Buffer A	3.0mL/min
Step 4:	25-30min	Buffer A	6.0mL/min

8.6. **Determine the HETP and h of the column per the BioLogic LP Chromatography System SOP.**

8.6.1. Use Method: IEX HETP

Step1:	0-15min	Buffer A	5mL/min

8.6.2. The Dp of the bead is 0.05mm.

8.6.3. The expected HETP is approximately 0.1mm.

8.6.4. The h calculation should be less than 3. If h is greater than 3, the desired product may not bind the column efficiently. In this case it is best to re-pack the column.

8.7. **Run Column per the BioLogic LP Chromatography System SOP.**

8.7.1. Place the lines for Buffers A, B and C into the vessels containing the appropriate buffers. Cover the opening of each vessel with laboratory film.

8.7.2. Use Method: IEX tPA

Step 1:	0 to 20min	Buffer C	4mL/min
Step 2:	20 to 40min	Buffer A	4mL/min
Step 3:	40 to 60min	Buffer B	4mL/min
Step 4:	60 to 80min	Buffer A	4mL/min

8.7.3. Collect 1-5mL of the flow through fraction when the first A.U. peak begins to plateau (approximately 5 minutes into the run).

8.7.4. Collect the entire elution fraction when the second A.U. peak BEGINS to appear (approximately 40 minutes into the run).

8.7.5. Store fractions at 2-8^{o}C for SDS PAGE analysis.

8.8. **Clean the Column per the BioLogic LP Chromatography System SOP.**

8.8.1. Place the lines for buffers A and B into the vessel containing Cleaning Solution, 0.1M NaOH. Cover the opening of the vessel with laboratory film.

Title: Ion Exchange Chromatography of tPA SOP

8.8.2. Use Method: IEX Clean

Step 1: 0 to 20min Buffer 50% B 4mL/min

8.9. **Store the System per the BioLogic LP Chromatography System SOP.**

9. Attachments: N/A

10. History:

Name	Date	Amendment
Sonia Wallman	2000	Initial Release
Deb Audino	7/2005	Changed from manual pump system to BioLogic LP system.
Deb Audino	051206	Removed steps associated with equipment operation to simplify the process SOP.
Deb Audino	31Aug07	Simplified the packing method.
Deb Audino	04Apr08	College name change
Kari Britt	31May09	Added labeling directions, directions for covering vessels with laboratory film, and directions for placing buffer lines into the appropriate buffer. Also made general grammar and formatting edits as needed throughout the document.

Title: BioLogic LP Chromatography System Operating SOP

Approvals:
Preparer: ___Kari Britt _____ Date:_____02Aug10_____
Reviewer: __Sonia Wallman_____ Date:_____02Aug10_____

1. **Purpose:** Operation of the BioLogic LP Chromatography System.
2. **Scope:** Applies to the BioLogic LP Chromatography System for purifying proteins.
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. BioLogic LP Chromatography System Instruction Manual
5. **Definitions**:
   5.1. CV: Column Volume; $CV = \pi(L \text{ in cm})[(\text{radius of column in cm})^2]$
   5.2. L = Length of column (meaning the height of the bead bed)
   5.3. HETP: Height Equivalent to Theoretical Plate; HETP = L/N
   5.4. $N = 5.54 (t_R/w_{1/2})^2$
   5.5. t_R: retention time
   5.6. $w_{1/2}$: peak width at half height
   5.7. h: Reduced Plate Height; h = HETP/Dp
   5.8. Dp: bead diameter
6. **Precautions**: N/A
7. **Materials:**
   7.1. deionized Water
   7.2. Equilibration Buffer A (Refer to the process SOP)
   7.3. Equilibration Buffer B (Refer to the process SOP)
   7.4. Cleaning Solution (Refer to the process SOP)
   7.5. biopure water
   7.6. container for waste fluid
   7.7. collection tubes for fraction collector or collection containers
   7.8. column (Amicon Vantage-L Biochromatography column and accessories)
   7.9. resin (Refer to the process SOP.)
   7.10. lab towels
8. **Procedure:**
   8.1. Turn on BioLogic LP system (switch is in the front, on the lower left side of the system).
   8.2. Turn on computer.
   8.3. Click on the LP DataView icon.
   8.4. Verify that the computer is communicating with the system as indicated by a green "Receive" circle on the upper right side of the computer screen.
   8.5. **Pump Calibration**

Title: BioLogic LP Chromatography System Operating SOP

8.5.1. Based on the desired flow rate, select the appropriate tubing for the pump as follows:
Flow rates of 0.04-0.8 mL/min require 0.8mm tubing.
Flow rates of 0.2-4.0 mL/min require 1.6mm tubing.
Flow rates of 0.8-15.0 mL/min require 3.2mm tubing.

8.5.2. Verify that the correct tubing is in the pump.
8.5.2.1. Remove the platen by lifting the grey handle (Figure 2).
8.5.2.2. If necessary, insert the correct tubing.
8.5.2.3. Replace platen and lock into place.
8.5.2.4. If tubing was replaced readjust the platen and recalibrate the pump.
8.5.2.4.1. Loosen the platen adjust screw located on the top of the pump (Figure 2) by turning counterclockwise until there is slight resistance.
8.5.2.4.2. Tighten the platen screw clockwise the appropriate number of COMPLETE turns.
0.8mm tubing requires 5 turns
1.6mm tubing requires 4 turns
3.2mm tubing requires 3 turns
8.5.2.5. Recalibrate the pump.
8.5.2.5.1. Press the MANUAL mode key.
8.5.2.5.2. Press the PUMP instrument key.
8.5.2.5.3. Select FLOW, then select CALIBRATE.
8.5.2.5.4. Select the appropriate tubing size.
8.5.2.5.5. Select NOMINAL.

8.6. **Purge System with Buffer A and Zero the UV Monitor**

8.6.1. Place each buffer line into a container filled with Buffer A (Equilibration Buffer).
8.6.2. Attach the column inlet tube directly to the column outlet tube.
8.6.3. Press the MANUAL mode key.
8.6.4. Select BUFFER.
8.6.5. Select MIX.
8.6.6. Type in 50% B.
8.6.7. Select OK.
8.6.8. Select PURGE.
8.6.9. Allow system to purge until conductivity reading on the display panel of the Biologic LP system controller stabilizes (less than 5 minutes).
8.6.10. Select BUFFER.
8.6.11. Using the arrow key, select C.
8.6.12. Select OK.
8.6.13. Allow system to purge until conductivity reading on the display panel of the controller stabilizes (less than 5 minutes).
8.6.13.1. While the system is running, zero the UV Monitor.
8.6.13.1.1. Press the UV instrument key.
8.6.13.1.2. Select ZERO.

Title: BioLogic LP Chromatography System Operating SOP

8.6.13.1.3. Verify that the absorbance changes to zero on the display panel of the controller.
8.6.13.1.4. Press the PUMP instrument key.
8.6.14. After conductivity stabilizes, select STOP.

8.7. **Pour the Column (if necessary)**
8.7.1. Secure the column in an upright position to a stand using clamps.
8.7.2. Close the bottom valve on the column (handle should be in the horizontal position).
8.7.3. Add approximately 10mL Buffer A to the column.
8.7.4. Obtain the appropriate resin (Refer to the process SOP.).
8.7.5. Swirl the resin to make a homogeneous mixture.
8.7.6. Measure the appropriate amount of resin (Refer to the process SOP.) with a graduated cylinder and transfer to the column.
8.7.7. Dislodge any beads that stick to the column with additional buffer.
8.7.8. Position the 3 way valve on the top adapter to close the top port (handle points to the top port).
8.7.9. Place the tubing from 3 way valve into the waste container.
8.7.10. Secure the adapter housing to the glass column.
8.7.11. Allow the resin to settle until there is a clear layer of buffer above the surface of the resin.
8.7.12. Depress the top adapter until it reached approximately 3cm above the resin, making sure air and then liquid comes out the top of the 3 way valve.
8.7.13. Lock the adapter into place.

8.8. **Attach the Column**
8.8.1. Position the 3 way valve to close off the column (handle points to the column).
8.8.2. Disconnect the column inlet and outlet tubing from the tubing connector.
8.8.3. Attach the column inlet tubing from the injector valve to the top of the column 3 way valve.
8.8.4. Attach the column outlet tubing to the bottom of the column.
8.8.5. Place tubing from the 3 way valve side port in the waste container.
8.8.6. Open the valve at the bottom of the column (handle in vertical position).
8.8.7. Press MANUAL mode key.
8.8.8. Select PURGE.
8.8.9. Allow buffer to drip into the waste container from the side port until air bubbles are completely absent from the tubing.
8.8.10. Simultaneously select STOP and position the 3 way valve to close the side port.

8.9. **Pack the Column**
8.9.1. Place all lines in the appropriate buffers/solutions as per the process SOP.
8.9.2. Press the PROGRAM mode key.
8.9.3. Select LIST METHODS.

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8.9.4. Using the arrow keys, select the correct method (Refer to the process SOP.). If the method is not listed, refer to the Biologic LP Chromatography System Instruction Manual to create a new program.
8.9.5. Select OPEN.
8.9.6. Using the arrow keys, verify that the method agrees with the process SOP.
8.9.6.1. If the method has been changed, refer to the Biologic LP Chromatography System Instruction Manual to edit the program.
8.9.7. Select DONE.
8.9.8. Press the RUN mode key.
8.9.9. System will have a 10 second delay.
8.9.10. Verify that the computer is recording data by the appearance of an S symbol on the graph.
8.9.10.1. If the S is not present, click the "record" button on the toolbar on the computer screen.
8.9.11. Once the method is finished, unlock the top adapter, lower the top adapter down to ~2mm above the beds and then re-lock the adapter.
8.9.11.1. Measure the bed height.
8.9.11.2. Determine the Column Volume for your column using the following formula: $CV = \pi(L \text{ in cm})[(\text{radius of column in cm})^2]$
Refer to Definitions (section 5) as needed to complete the calculation.
8.9.11.3. There is no need to save the chromatogram, clear the screen using the "clear" button on the toolbar.

8.10. **Determine the HETP and h**
8.10.1. Attach an appropriate size sample loop to the sample valve (usually a 125µL loop is appropriate).
8.10.2. Turn MV-6 injector valve knob counterclockwise until there is resistance.
8.10.3. Draw 1mL of elution buffer into a syringe.
Note: If the elution buffer does not contain salt, then sterile filter a 1M sodium chloride solution to inject into the system.
8.10.4. Insert syringe into top port. Push slowly to fill sample loop while simultaneously collecting overflow in a beaker.
8.10.5. Leave syringe in port.
8.10.6. Turn MV-6 injector valve knob clockwise until there is resistance.
8.10.7. Press the PROGRAM mode key.
8.10.8. Select LIST METHODS.
8.10.9. Using the arrow keys, select the correct method (refer to the process SOP). If the method is not listed, refer to the Biologic LP Chromatography System Instruction Manual to create a new program.
8.10.10. Select OPEN.
8.10.11. Using the arrow keys, verify that the method agrees with the process SOP.
8.10.11.1. If the method has been changed, refer to the Biologic LP Chromatography System Instruction manual to edit the program.
8.10.12. Select DONE.

Title: BioLogic LP Chromatography System Operating SOP

8.10.13. Press the RUN mode key.
8.10.14. System will have a 10 second delay.
8.10.15. Verify that the computer is recording data by the appearance of an S symbol on the graph.
8.10.15.1. If the S symbol is not present, click the "record" button on the toolbar on the computer screen.
8.10.16. Once a full peak has been generated, stop the program.
8.10.17. Turn MV-6 injector valve knob counterclockwise until there is resistance.
8.10.18. Save and print the file, making note of the directory where the chromatogram was saved.
8.10.19. Clear the screen.
8.10.20. From the chromatogram determine the HETP and h.

HETP = L/N

Note: Use L in mm for this calculation.

$N = 5.54 (t_R/w_{1/2})^2$

h = HETP/Dp

Refer to Definitions (section 5) and Figure 5 as needed to complete the calculations. Refer to the process SOP for the Dp value.

8.11. **Run the Column**
8.11.1. Place all lines in the appropriate buffers/solutions as per the process SOP.
8.11.2. Press the PROGRAM mode key.
8.11.3. Select LIST METHODS.
8.11.4. Using the arrow keys, select the correct method as per the process SOP. If the method is not listed, refer to the Biologic LP Chromatography System Instruction Manual to create a new program.
8.11.5. Select OPEN.
8.11.6. Using the arrow keys, verify that the method has not been changed.
8.11.6.1. If the method has been changed, refer to the Biologic LP Chromatography System Instruction Manual to edit the program.
8.11.7. Select DONE.
8.11.8. Press the "Run" mode key.
8.11.9. System will have a 10 second delay.
8.11.10. Verify that the computer is recording data by the appearance of an S symbol on the graph.
8.11.10.1. If the S symbol is not present, click the "record" button on the toolbar on the computer screen.

8.12. **Clean the Column**
8.12.1. Place buffer lines into the appropriate cleaning solution (Refer to the process SOP.).
8.12.2. Run the appropriate cleaning method (Refer to the process SOP.).

8.13. **Clean and Store the System**
8.13.1. If the system will be used again with the same column within a few days, it may be stored "as is" after a run.

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8.13.1.1. Turn off the system and turn off the computer.

8.13.2. If the system will not be used within a few days it must be flushed with water then 20% ethanol and purged with air.

8.13.2.1. Disconnect the column.
8.13.2.2. Attach the column inlet tube directly to the column outlet tube.
8.13.2.3. Place each buffer line into a container filled with biopure water.
8.13.2.4. Attach the column inlet tube directly to the column outlet tube.
8.13.2.5. Press the MANUAL mode key.
8.13.2.6. Select BUFFER, then select MIX.
8.13.2.7. Type in 50% B, then select OK.
8.13.2.8. Select PURGE.
8.13.2.9. Allow system to purge until conductivity reading stabilizes (less than 5 minutes).
8.13.2.10. Select BUFFER.
8.13.2.11. Using the arrow key, select C.
8.13.2.12. Select OK.
8.13.2.13. Allow system to purge until conductivity reading stabilizes (less than 5 minutes).
8.13.2.14. Select STOP.
8.13.2.15. Place each buffer line into 20% Ethanol and repeat steps 8.13.2.6. through 8.13.2.16.
8.13.2.16. Place each buffer line on a lab towel or kimwipes so that they are open to the air and repeat steps 8.13.2.6. through 8.13.2.16.
8.13.2.17. Turn off the LP Biologic System.

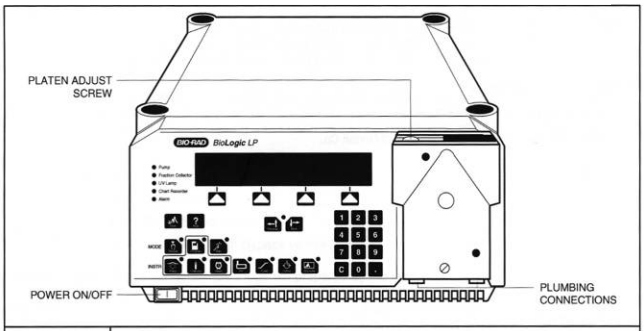
9. Attachments:

9.1. Figure 1: Controller Front Panel
9.2. Figure 2: Controller Pump
9.3. Figure 3: LP Biologic System Parts
9.4. Figure 4: Column components
9.5. Figure 5: Chromatogram example for calculating HETP

10. History:

Name	Date	Amendment
Deb Audino	070105	Initial release
Deb Audino	110405	Removed purging the system with water and Buffer B prior to use. Added the cleaning and storing section.
Deb Audino	17May06	Added the column components figure, added steps that were removed from the process SOPs.
Bob O'Brien	23Jan08	Added steps to clarify use of the 3 way valve.
Deb Audino	04Apr08	College name change
Kari Britt	03Aug10	Added to definitions and HETP sections. Added Figure 5. Made grammar and formatting edits as needed throughout the document. Removed references to programming SOP.

Title: BioLogic LP Chromatography System Operating SOP



Feature	Description
Control Panel	Consists of the control keys and status LEDs for monitoring and controlling the system. It is designed to withstand the minor spills associated with use in a laboratory.
Power switch	Turns on/off the BioLogic LP Controller.
Plumbing Connections	The peristaltic pump may be used with most flexible tubing having an inner diameter less than or equal to 3.2 mm (1/8") and a wall thickness of 1.0 mm or less, including PharMed, and silicone. Inlet and outlet lines attach to the ports at the bottom of the pump. These ports accept standard luer fittings.

Figure 1: Controller Front Panel

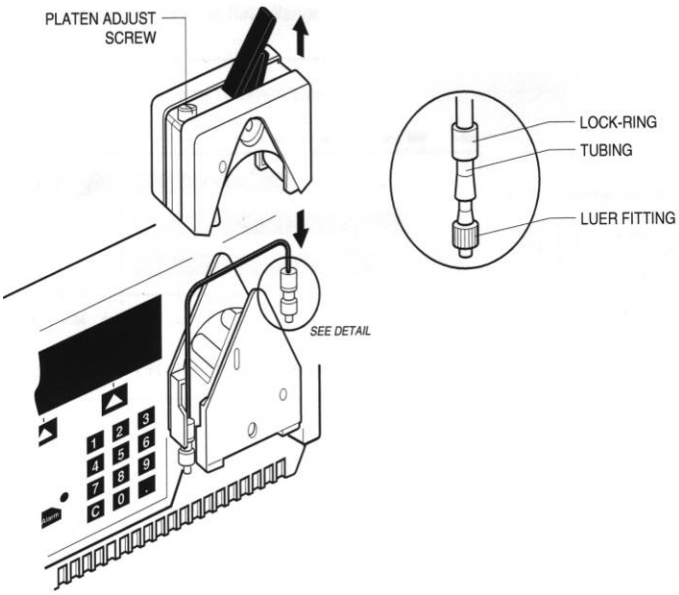


Figure 2: Controller Pump

Title: BioLogic LP Chromatography System Operating SOP

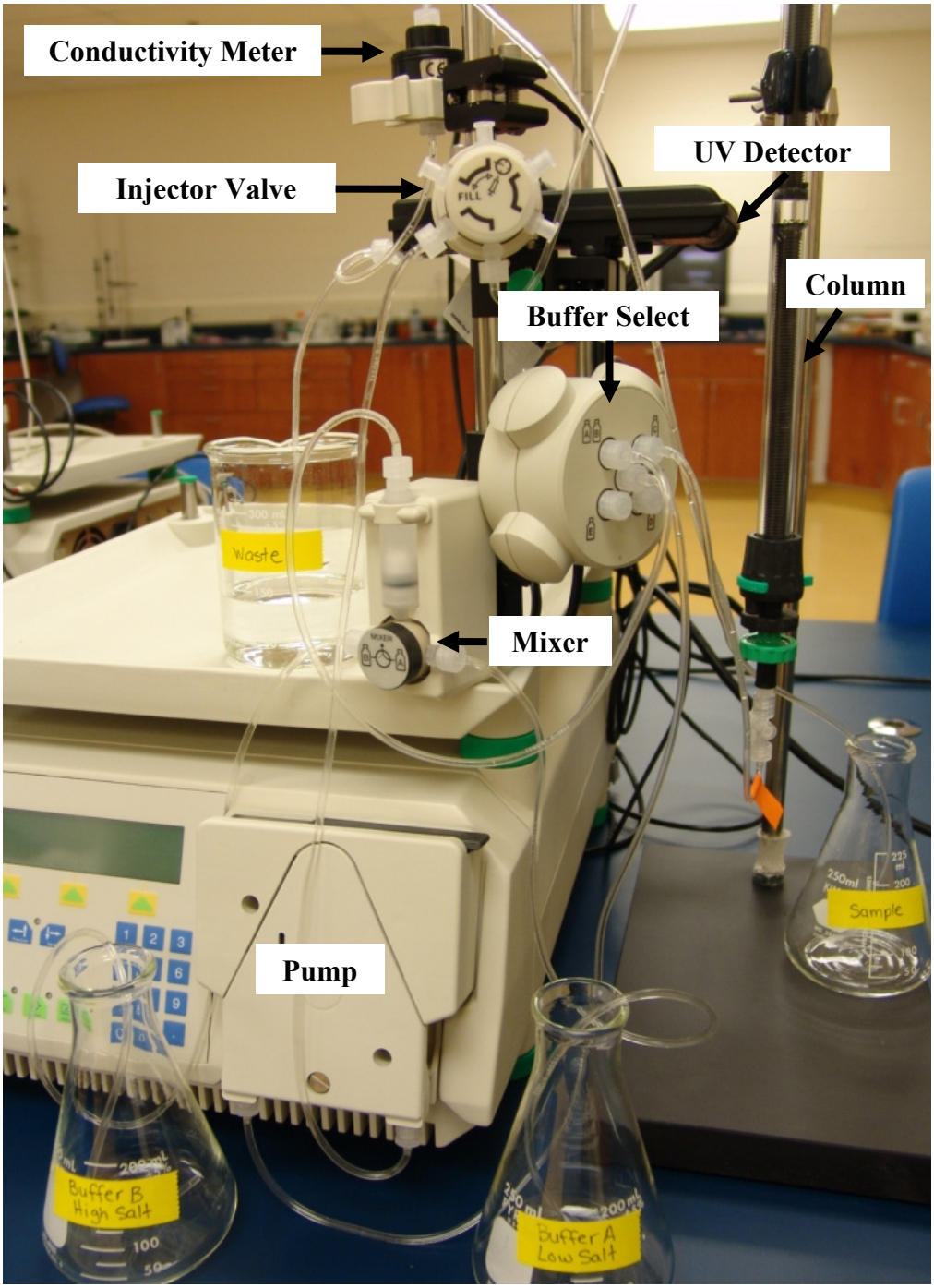


Figure 3: LP Biologic System Parts

Title: BioLogic LP Chromatography System Operating SOP

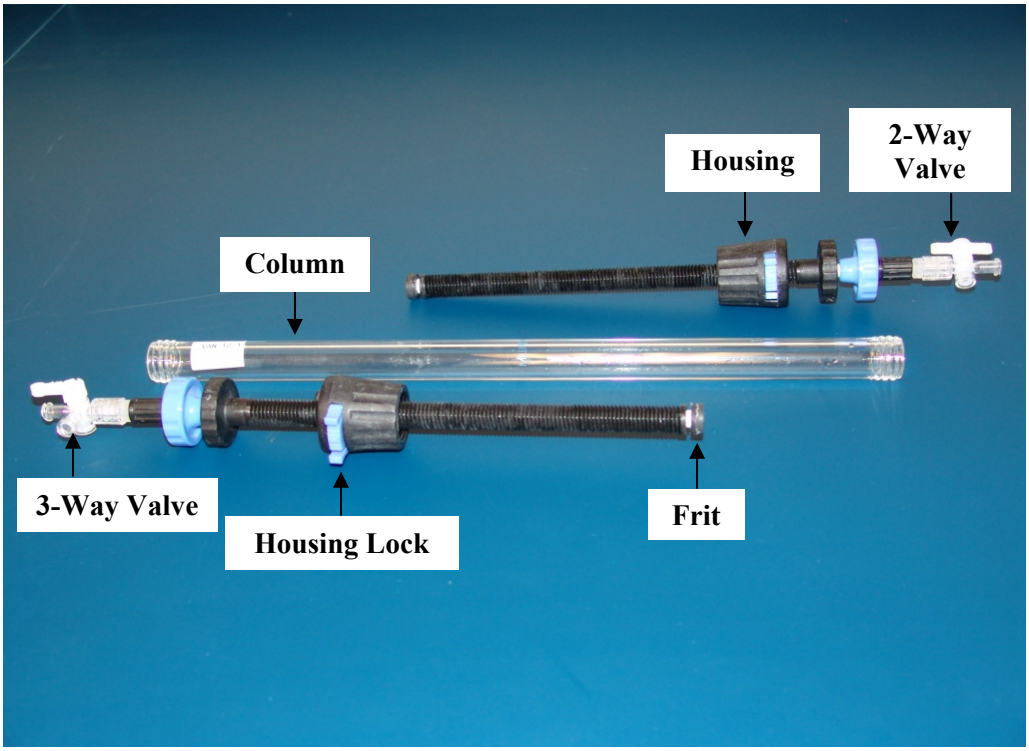


Figure 4: Column Components

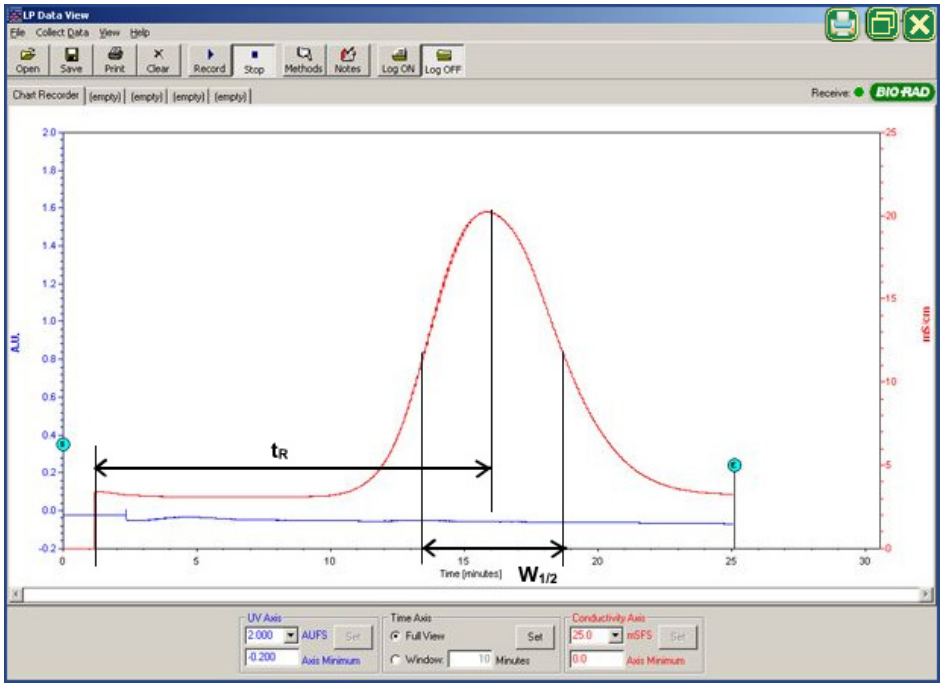


Figure 5: Chromatogram Example for Calculating HETP

Batch Record: tPA Production from CHO Cells Downstream 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 Downstream Process
tPA Lot Number_____

1. Solution and Buffer Preparation Buffer A: Equilibration Buffer, 20mM phosphate, pH 6. Buffer B: Elution Buffer, 20mM phosphate pH 6, 1M NaCl Cleaning Solution: 0.1M NaOH Adjust cell culture supernatant to pH 6.		
Calibrate pH meter per SOP with commercially prepared standard buffers (pH 7 and pH 4): pH Meter ID #_____ pH 7 Buffer Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ pH 4 Buffer Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____	Operator/Date	Verifier/Date
Weigh 2.10 ±0.05 grams of sodium phosphate monobasic anhydrous. Balance ID #:_____ Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Weigh 0.66 ±0.02 grams of sodium phosphate dibasic heptahydrate. Balance ID #:_____ Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Dissolve sodium phosphate monobasic anhydrous with the sodium phosphate dibasic heptahydrate in approximately 1L of deionized water using magnetic stir bar. Volume of water added _____ mL	Operator/Date	Verifier/Date
Adjust Equilibration Buffer A to pH 6.0 ±0.1. pH_____	Operator/Date	Verifier/Date
Sterile filter solution and label as: Buffer A, Equilibration Buffer, 20mM Phosphate, pH 6, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Downstream Process
tPA Lot Number_____

Weigh 29.2 ±0.2 grams NaCl. Balance ID #:_____ Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Dissolve in approximately 500ml of Equilibration Buffer A using magnetic stir bar. Volume of Buffer A added _____ mL	Operator/Date	Verifier/Date
Sterile filter solution and label as: Buffer B, Elution Buffer, 20mM Phosphate, pH 6, 1M NaCl, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].	Operator/Date	Verifier/Date
Weigh 2.0 ±0.05 grams of NaOH. Balance ID #:_____ Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Dissolve in approximately 500ml deionized water using magnetic stir bar. Volume of water added _____ mL	Operator/Date	Verifier/Date
Sterile filter solution and label as Cleaning Solution, 0.1M NaOH, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].	Operator/Date	Verifier/Date
Adjust pH of CHO cell culture supernatant to pH 6 ±0.1. pH_____	Operator/Date	Verifier/Date
Label CHO cell culture supernatant as: Buffer C, CHO Cell Culture Supernatant, pH 6, Store: 2-8°C, Dispose: drain, [date], [group], [initials].	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Downstream Process
tPA Lot Number_____

2. Purge BioLogic LP System, Pour Column and Attach to Biologic LP System		
Calibrate pump if necessary per the BioLogic LP Chromatography System SOP. **Verify** that **1.6mm** tubing is in the pump. Change tubing if necessary. Tubing changed: Yes / No (Circle one.) If the tubing was changed, adjust the platen and calibrate the pump per BioLogic LP SOP. Platen adjusted: Yes / No (Circle one.) Pump recalibrated: Yes / No (Circle one.)	Operator/Date	Verifier/Date
Purge the BioLogic LP system with Buffer A per the Biologic LP Chromatography System SOP.	Operator/Date	Verifier/Date
Place each buffer line into a container filled with Buffer A (Equilibration Buffer).	Operator/Date	Verifier/Date
Zero the UV monitor per the Biologic LP Chromatography System SOP.	Operator/Date	Verifier/Date
Add approximately 5ml of POROS 50 HS resin to column per BioLogic LP Chromatography System SOP. Volume of POROS 50 HS added: _____ mL Amicon Vantage-L-Column ID#:_____	Operator/Date	Verifier/Date
Attach the column to the BioLogic LP per the BioLogic LP Chromatography System SOP. BioLogic LP ID#:_____	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Downstream Process
tPA Lot Number_____

3. Pack the Column and Determine HETP and h		
Pack column per the BioLogic LP Chromatography System SOP using Method: IEX Pack.	Operator/Date	Verifier/Date
Place the line for Buffer A into the vessel containing Buffer A, Equilibration Buffer. Cover the opening of the vessel with laboratory film, such as Parafilm.	Operator/Date	Verifier/Date
Determine column volume per the BioLogic LP Chromatography System SOP. CV = π(bed height in cm)(radius of column in cm)^2 Write out CV calculation in this space: Bed Height: _____ Column Volume: _____	Operator/Date	Verifier/Date
Produce chromatogram needed to determine HETP and h per BioLogic LP Chromatography System SOP using Method: IEX HETP. Volume of 0.8M NaCl loaded_____mL	Operator/Date	Verifier/Date
Determine HETP of the column per BioLogic LP Chromatography System SOP and **attach** chromatogram to batch record. Dp = 0.05mm for POROS HS resin. Write out HETP and h calculations in this space: HETP value:_____mm h value:_____	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Downstream Process
tPA Lot Number_____

4. Run Column		
Run column per the BioLogic LP Chromatography System SOP using Method: IEX tPA.	Operator/Date	Verifier/Date
Place the lines for Buffers A, B and C into the vessels containing the appropriate buffers. Cover the opening of each vessel with laboratory film.	Operator/Date	Verifier/Date
Store fractions at 2 – 8ºC for SDS PAGE Analysis.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date
5. Clean and Store BioLogic LP Chromatography System		
Clean the column per the BioLogic LP Chromatography System SOP using Method: IEX Clean.	Operator/Date	Verifier/Date
Place the lines for buffers A and B into the vessel containing Cleaning Solution, 0.1M NaOH. Cover the opening of the vessel with laboratory film.	Operator/Date	Verifier/Date
Clean and store the BioLogic LP Chromatography System per the BioLogic LP Chromatography System SOP. Column Storage (Check one): Left on Biologic System _____ Disconnected and stored at room temp. _____ Disconnected and stored at 2-8C _____ Disassembled _____	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Manufacturing Technician (Downstream)

REF	Key Functions & Tasks (Downstream Manufacturing Technician)
1	**Work in compliance with EH&S.**
1.a	Wear appropriate personal protective equipment.
1.b	Work in controlled environments.
1.c	Participate in emergency drills and emergency response teams.
1.d	Identify unsafe conditions and take corrective action.
1.e	Appropriately and safely access production equipment.
1.f	Handle, label, and dispose of hazardous / biohazard materials.
1.g	Access and utilize MSDS.
1.h	Perform permitting procedures.
1.i	Carries out operations with attention to OSHA and EPA regulations, and other applicable state and federal regulations.
1.j	Keeps work areas clean and safety equipment in order.
1.k	Participate in all company safety training and audits as required.
1.l	Assists with waste treatment operations.
2	**Work in compliance with cGMPs.**
2.a	Assists in environmental monitoring activities.
2.b	Follow SOPs for all operations.
2.c	Records process data and completes batch records as required.
2.d	Maintain equipment logbooks.
2.e	Control and receipt of raw materials.
2.f	Maintain training documentation.
2.g	Maintain equipment and processes in a validated state.
2.h	Working in controlled/classified areas (gowning, aseptic technique).
2.i	Ensure appropriate flow of personnel, equipment, and materials.
2.j	Change control for process, equipment, and documentation.
2.k	Label and apply status to equipment and materials.
2.l	Identify and report exception events and CAPA.
3	**Clean and maintain production areas.**
3.a	Housekeeping / pest control.
3.b	Sanitize and clean of controlled spaces.
3.c	Preparation of cleaning materials and solutions.
3.d	Assist in environmental monitoring for routine and changeover operations.
3.e	Document cleaning.
4	**Maintain effective communication.**
4.a	Deliver shift change update.
4.b	Communicate with coworkers and/or customers to ensure production or service meets requirements.
4.c	Suggest continuous improvements.
4.d	Coordinate with work teams / internal customers.
4.e	Maintain security and confidentiality.
4.f	Respond appropriately to internal auditors and external inspectors.
4.g	Assist in writing, reviewing, and commenting on technical documents.

REF	Key Functions & Tasks (Downstream Manufacturing Technician)
5	**Prepare process materials.**
5.a	Weigh, dispense, and label raw materials for use in production.
5.b	Dispense consumables and intermediates.
5.c	Control and reconcile inventory with enterprise control system (MRP, SAP, manual database).
5.d	Prepare and sterilize buffers and solutions.
5.e	Sample and test buffers and solutions.
5.f	Transfer buffers and solutions to use point.
5.g	Prepare filters for use.
5.h	Prepare, pasteurize / sterilize, and titrate media and feed solutions.
5.i	Manage chromatography resins.
6	**Prepare equipment.**
6.a	Clean CIP vessels, transfer lines, and filter trains .
6.b	Clean COP equipment (or sonicator).
6.c	Depyrogenate components and equipment.
6.d	Sterilize SIP vessels, transfer lines, and sampling ports.
6.e	Perform pressure test.
6.f	Prepare and assemble components and equipment.
6.g	Autoclave components and equipment.
6.h	Perform WFI flush of transfer lines.
6.i	Perform scheduled sanitizations of hoods.
6.j	Prepare and standardize probes and ancillary instruments.
6.k	Prepare, assembly, and integrity test filters.
6.l	Visually inspect equipment.
6.m	Maintain equipment logs and status tags.
6.n	Complete, review and approve equipment process records.
7	**Perform basic manufacturing operations.**
7.a	Perform processes following batch records, validation protocols, and/or SOPs.
7.b	Maintains and controls processes in an automated control environment.
7.c	Record process data.
7.d	Inspect materials at all stages of process to determine quality or condition.
7.e	Participate in the installation, modification, and upgrade of equipment.
7.f	Operate, monitor, and maintain equipment, tools, and workstation.
7.g	Recognize and respond appropriately to atypical events.
7.h	Participate in troubleshooting and root cause analysis of operations.
8	**Perform downstream manufacturing operations.**
8.a	Receive material from upstream processing.
8.b	Separate cells from media using centrifugation or filtration (TFF or depth filtration).
8.c	Perform cell disruption techniques (mechanical or chemical).
8.d	Perform aqueous separations (liquid liquid extraction, precipitation).
8.e	Perform chemical/enzymatic modifications to product.
8.f	Perform normal flow and tangential flow filtration (including microfiltration, ultrafiltration, or diafiltration).
8.g	Prepare chromatography columns (assembly, sanitization, resin packing, evaluation).
8.h	Perform chromatography steps (equilibrate, load, wash, elute, clean, store).
8.I	Perform viral clearance (removal/inactivation) steps.
8.j	Bulk fill purified product.
9	**Perform Sampling.**
9.a	Prepare sample port for aseptic sampling.
9.b	Obtain in-process samples according to batch records or sampling plans.
9.c	Label samples appropriately.
9.d	Record sample collection and distribution (storage and chain of custody)
9.e	Perform in-process chemical and/or microbiological tests.

Title: SDS-PAGE SOP

Approvals:
Preparer:_____Deb Audino_____Date ____03Apr08_____
Reviewer:_____Kari Britt_____Date ____03Apr08_____

1. **Purpose:**
   1.1. To describe the appropriate operating instructions to perform SDS PAGE analysis of proteins samples.
2. **Scope:**
   2.1. Applies to confirming the presence and purity of the two human proteins (tPA and HSA) we have produced and purified in this class.
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. Invitrogen Novex Gel instructions
   4.2. Novex XCell II Mini-Cell Gel Box Operation SOP
   4.3. gel documentation instrument SOP
5. **Definitions:** N/A
6. **Precautions:**
   6.1. Acrylamide is a neurotoxin. Always wear protective gloves when handling the polyacrylamide gels.
   6.2. Fixative Solution is acidic and flammable. Keep it away from sparks and flames. Dispose in Fixative Hazardous Waste bottle
   6.3. GelCode Blue is harmful. Dispose in GelCode Blue Harzardous Waste bottle.
7. **Materials:**
   7.1. protein samples
   7.2. protein standard, 4mg/mL
   7.3. molecular weight marker (SeeBlue® Plus 2 Pre-stained Standard by Invitrogen is recommended. Catalog number: LC 5925)
   7.4. NOVEX Precast Gel Box and accessories
   7.5. power supply for protein electrophoresis
   7.6. NuPAGE 4-12% Bis-Tris Gels (1.0mm x 10 well)
   7.7. NuPAGE MOPS SDS Running Buffer (20X)
   7.8. NuPAGE Antioxidant
   7.9. NuPAGE SDS Sample Buffer (4X)
   7.10. reducing agent (10X)
   7.11. graduated cylinders (100mL, 250mL, 1L)
   7.12. P20, P100 or P200 Micropipettor and tips, including gel loading tips
   7.13. microfuge Tubes
   7.14. microfuge
   7.15. boiling water bath
   7.16. staining trays
   7.17. rotary shaker

Title: SDS-PAGE SOP

7.18. Fixative Solution
7.19. Pierce GelCode Blue Staining Reagent
7.20. light box
7.21. gel documentation instrument

8. Procedure:

8.1. **Prepare Running Buffers and Fixative Solution if needed.**

8.1.1. **Lower Buffer:** 1X NuPAGE MOPS SDS Running Buffer (1Liter)

8.1.1.1. Place 50mL of 20X NuPAGE MOPS SDS Running Buffer in a 1 Liter graduated cylinder.

8.1.1.2. Gently add 950mL deionized water by running it down the side of the cylinder to make 1 liter of 1X NuPAGE MOPS SDS Running Buffer.

8.1.1.3. Add a stir bar and gently stir.
Note: SDS is a detergent and will foam if mixed vigorously.

8.1.2. **Upper Buffer:** 1X NuPAGE MOPS SDS Running Buffer plus antioxidant (200mL)

8.1.2.1. Add 200mL of 1X NuPAGE MOPS SDS Running Buffer to an appropriate vessel.

8.1.2.2. Add 500µL of NuPAGE Antioxidant.

8.1.2.3. Add a stir bar and gently stir.
Note: SDS is a detergent and will foam if mixed vigorously.

8.1.3. **Fixative Solution** (500mL)

8.1.3.1. In a 500mL bottle, mix together:
250mL 100% Methanol
215mL deionized water
35mL glacial acetic acid

8.1.3.2. Store at 2– 8°C until needed.

8.2. **Prepare Protein Samples.**

Note: Do NOT perform this step with the Molecular Weight Marker.

8.2.1. For all the samples and the standards, combine the following in a sterile microfuge tube:
25µL 4x sample buffer
10µL 10x reducing agent
65µL sample

8.2.2. Mix gently with a pipet by aspirating and dispensing at least 3 times.

8.2.3. Boil for 3-5 minutes.

8.2.4. Remove from boiling water bath.

8.2.5. Pulse all samples and standards in a microfuge for 30 seconds.

8.3. **Prepare Novex Precast Gel Box.**

8.3.1. Assemble gel box according to its SOP.

8.3.2. Place 200mL NuPAGE MOPS SDS Running Buffer (1X) plus antioxidant in the upper buffer chamber (small chamber between 2 gels or the gel and buffer dam).

8.3.3. Fill the lower buffer chamber with approximately 600mL of 1X NuPAGE MOPS SDS Running Buffer (large chamber).

Title: SDS-PAGE SOP

8.4. **Load Samples.**

8.4.1. Using a micropipettor and disposable tips, load 10µL of the Molecular Weight Marker into one well and up to 50µL of each sample into separate wells.

8.4.1.1. Avoid loading samples symmetrically.

8.4.2. Load any empty wells with 15µL of diluted 4X Sample Buffer.

8.4.3. Record order of samples and volumes loaded.

8.5. **Run NOVEX NuPAGE MOPS SDS Precast Gel Box.**

8.5.1. Plug electrophoresis chamber into the gel electrophoresis power supply.

8.5.2. Run gel at 200V for 40 – 60 minutes.

8.5.3. Turn off the power supply when the dye reaches 1cm from the bottom of the gel.

8.6. **Stain and Photodocument the NOVEX NuPAGE MOPS SDS Precast Gel.**

8.6.1. Disassemble gel box per SOP and remove gel from plastic cassette.

8.6.2. Rinse gel box well with DI water. Do not use brushes on the gel box, they scratch the surface. Do not immerse top of gel box or electrical components.

8.6.3. Place gel in staining tray.

8.6.4. Wash gel 3 times for approx. 5 minutes with DI water shaking at room temp.

8.6.5. Add enough Fixative solution to completely cover the gel and fix for approx. 15 minutes shaking at room temp.

8.6.6. Discard Fixative Solution into the Fixative Hazardous Waste bottle

8.6.7. Wash gel 3 times for a minimum of 5 minutes with DI water shaking at room temp.

8.6.8. Add about 50mL of GelCode Blue and stain for 1-24 hours shaking at room temp.

8.6.9. Decant GelCode Blue into GelCode Blue Hazardous Waste bottle.

8.6.10. Wash gel with DI water for 15 minutes to overnight on a shaker

8.6.11. Remove gel from staining tray and place on visible light box

8.6.12. Identify the protein standards and samples and estimate their molecular weights. See Molecular Weight Diagram.

9. Attachments:

9.1. Figure 1: Molecular Weight Marker Diagram

10. History:

Name	Date	Amendment
Sonia Wallman	2000	Initial Release
SCP	2003	Changed Coomassie stain to GelCode Blue Stain
Deb Audino	2005	Put into SOP 2005 format
Deb Audino	09May06	Removed dilute protein standards
Deb Audino	04Apr08	College name change

Title: SDS-PAGE SOP

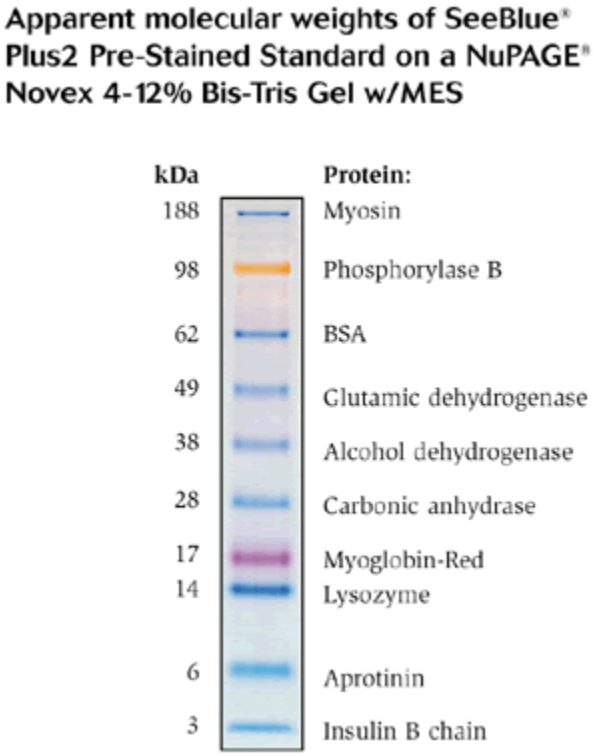


Figure 1: Molecular Weight Marker Diagram

Title: Xcell *SureLock* Mini-Cell Gel Box SOP

Approvals:
Reviewer: __ Bob O'Brien _____ Date__08Apr08_____
Reviewer: __ Deb Audino_____Date__08Apr08_____

1. **Purpose:**
   1.1. Assembly and disassembly of the XCell *SureLock* ™ mini-cell gel box.
2. **Scope:**
   2.1. Applies to the assembly and disassembly of the XCell *SureLock*™ mini-cell gel box for use in SDS PAGE.
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. XCell *SureLock* ™ Mini-Cell Gel Box Instruction Manual.
5. **Definitions:** N/A
6. **Precautions:**
   6.1. Do not attempt to use the XCell *SureLock*™ mini-cell gel box without the XCell *SureLock* lid.
   6.2. Maximum voltage limit: 500 VDC
   6.3. Maximum power limit: 50 Watts
   6.4. Maximum operating temperature limit: 70°C
   6.5. Acrylamide is a neurotoxin. Always wear protective gloves when handling the polyacrylamide gels.
7. **Materials:**
   7.1. pre-cast gel cassette
   7.2. D.I. (deionized) water
   7.3. running buffer
   7.4. external power supply
   7.5. XCell *SureLock* ™ Mini-Cell
   7.6. buffer core with electrodes
   7.7. cell safety lid with power cords
   7.8. gel tension wedge
   7.9. buffer dam
   7.10. gel knife
8. **Procedure:**
   8.1. **Assembly of the Gel Box**
      8.1.1. Lower the buffer core into the lower buffer chamber so that the negative electrode fits into the opening in the brass plate.
      8.1.2. Cut open gel cassette pouch with scissors, drain away and dispose of the gel-packaging buffer.

Title: Xcell *SureLock* Mini-Cell Gel Box SOP

8.1.3. Handling cassette by its edges only, remove the gel cassette from the pouch and rinse with D.I. water.

8.1.4. **Peel off the tape** covering the slot on the back of the gel cassette.

8.1.5. In one fluid motion, carefully remove comb from cassette.
Note: Do not twist comb, pull straight out or damage to wells may occur.

8.1.6. Use a pipette to gently wash the cassette wells with running buffer, invert the gel and shake gently to remove buffer. Repeat twice.

8.1.7. Fill the sample wells with running buffer. Be sure to remove any bubbles from cassette wells.

8.1.8. Insert the gel cassette into the lower buffer chamber to create the upper buffer chamber.

8.1.8.1. If running only one gel, place the gel in front of the buffer core with the shorter (notched) side of the cassette facing in toward the core. Place the buffer dam behind the core. **Do not discard buffer dam.**

8.1.8.2. If running two gels, place one cassette in front of the buffer core and one cassette behind the core, making sure that the shorter (notched) sides are facing in towards the core.

8.1.9. Slide Gel Tension Wedge into the lower buffer chamber behind the buffer dam (or behind second gel) with the tapered end pointing up. See Figure 2.

8.1.10. Pull forward (toward the front of the unit) on the Gel Tension Lever until lever comes to a firm stop and the gels or gel/buffer dam appear snug against the buffer core. See Figure 2.

8.2. **Run the gel.**

8.2.1. Load and run the gel per the SDS-PAGE SOP

8.3. **Disassembly of the XCell *SureLock*™ Mini-Cell Gel Box**

8.3.1. Upon completion of the run, turn off the power and disconnect the electrode cords from the power supply.

8.3.2. Remove the lid.

8.3.3. Unlock the Gel Tension Lever by pushing the lever toward the back of the unit.

8.3.4. Remove gel cassette from the assembly. Handle gel cassette by the edges.

8.3.5. Lay the gel cassette on top of a lab towel, with the shorter plate on top. Allow one side to hang approximately 1 cm over the side of the bench top.

8.3.6. Insert the gel knife between the two plates. See **Figure 3**. (HINT: It may be easier to start with the corner.)

8.3.7. Twist the handle to separate the plates. You will hear a cracking sound which means you have broken the bonds which hold the plates together.

8.3.7.1. Do not push the knife forcefully between the cassette plates or the gel may be cut into and damaged.

8.3.8. Rotate the cassette and repeat steps 8.3.6. and 8.3.7. until the two plates are completely separated.

8.3.9. Using hands only and being very careful not to rip the gel, gently remove and discard the top plate, allow the gel to remain on the bottom plate.

Title: Xcell *SureLock* Mini-Cell Gel Box SOP

8.3.10. Holding the cassette plate over a container with the gel facing downwards gently push the gel knife into the slot at the bottom of the cassette, until the gel peels away from the plate.
8.3.11. If the gel is not easily removed, rinse with D.I. water from a squirt bottle inserted gently between the plate and the gel.
8.3.12. Cut the lip off the bottom of the gel (If needed).
8.3.13. Discard running buffer and rinse gel box well with deionized water. Do not use brushes. Do not immerse top of gel box or electrical components.

8.4. **Stain the gel.**
8.4.1. Stain the gel per the SDS-PAGE SOP.

9. Attachments:
9.1. Figure 1: Gel Box Parts
9.2. Figure 2: Assembled Gel Box Side View
9.3. Figure 3: Opening a Gel Cassette

10. History:

Name	Date	Amendment
Katrice Jalbert	030106	Initial Release
Bob O'Brien	08Apr08	Update the date format, change college name and remove outline of text boxes.

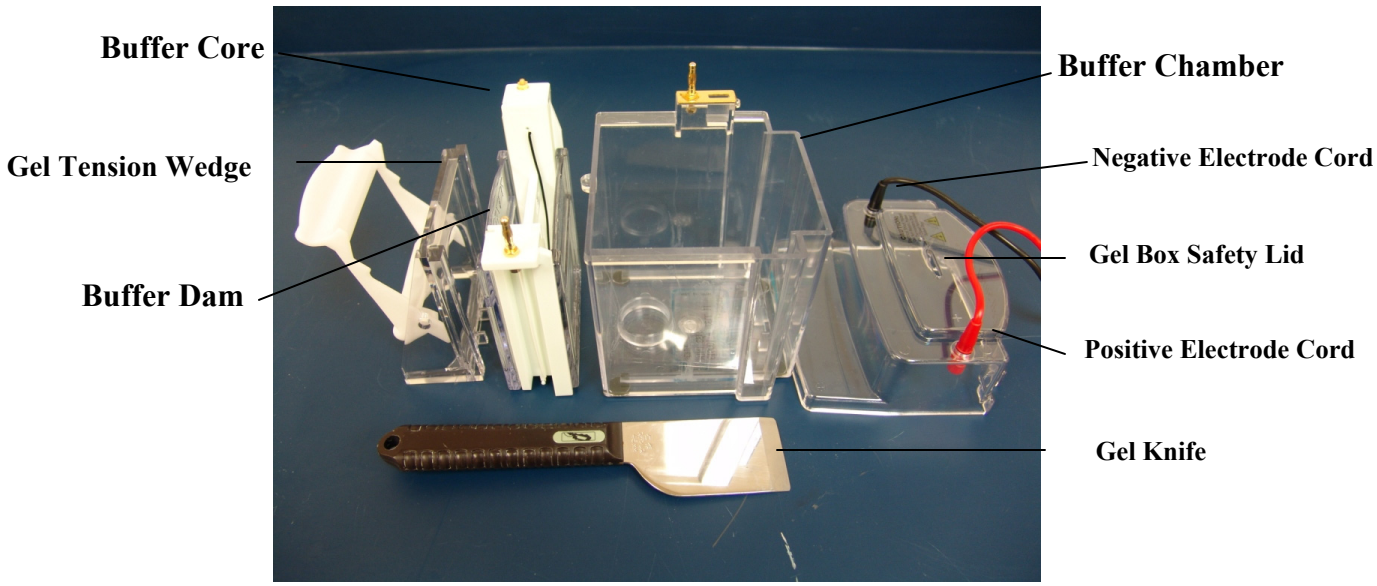


Figure 1: Gel Box Parts

Title: Xcell *SureLock* Mini-Cell Gel Box SOP

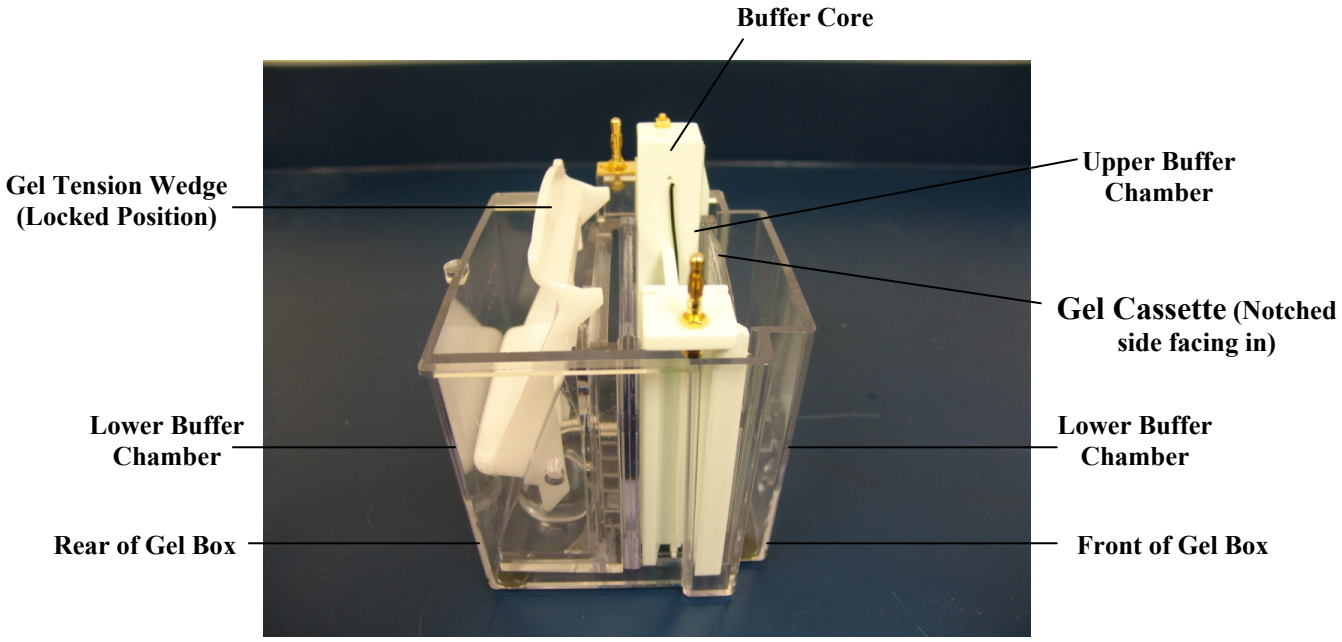


Figure 2: Assembled Gel Box Side View

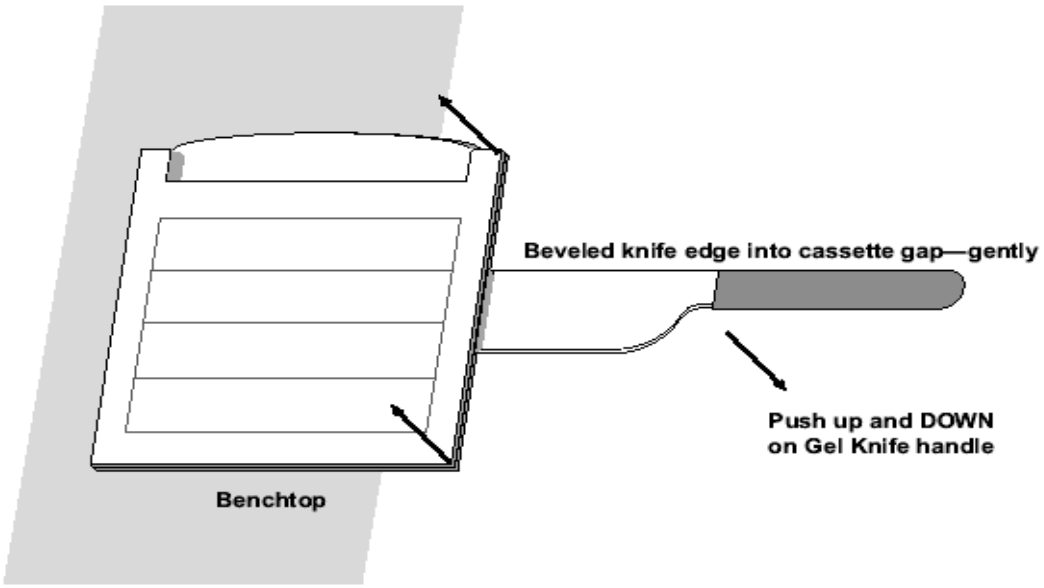


Figure 3: Opening a Gel Cassette

Title: tPA ELISA SOP

Approvals:
Preparer:_____Kari Britt_____ Date _____ 09Jun09_____
Reviewer:_____Bob O'Brien_____ Date _____ 09Jun09_____

1. **Purpose:**
   1.1. To determine the concentration of tPA in a sample.
2. **Scope:**
   2.1. Applies to determining the concentration of tPA in a sample using the IMUBIND tPA ELISA kit from American Diagnostica.
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. IMUBIND tPA ELISA kit instructions
   4.2. plate reader SOP
5. **Definitions:** N/A
6. **Precautions:**
   6.1. Plasma is of human origin and should be treated as Biosafety Level 2. Dispose of waste in biohazard containers.
7. **Materials:**
   7.1. IMUBIND tPA ELISA kit from American Diagnostica (cat# 860)
      7.1.1. Microtest strips coated with anti-tPA IgG coat and non- immune IgG
      7.1.2. PET buffer
      7.1.3. tPA depleted plasma
      7.1.4. tPA antigen standard plasma
      7.1.5. Conjugate -HRP labeled anti-tPA Fab fragments
   7.2. TMB (3, 3', 5, 5' – Tetramethylbenzidine) substrate
   7.3. disposable reagent reservoirs or weigh boats
   7.4. micropipettors (P-20, P-100 or 200, multichannel) and tips
   7.5. lab towels
   7.6. microfuge tubes
   7.7. microtiter plate reader operable at 630nm
8. **Procedure:**
   8.1. **Sample and Reagent Preparation**
      8.1.1. **PET buffer:**
         8.1.1.1. Dissolve the contents of the PET-buffer vial in 1L ± 50mL of water.
         8.1.1.2. Stir until dissolved, approximately 15 minutes.
         8.1.1.3. Label as: PET buffer, Store: 2-8°, Dispose: Drain, [Date], [Inititials].
      8.1.2. **Prepare Detection Antibody Conjugate:**
      8.1.3. Dilute the 100X Detection Antibody Conjugate with PET buffer according to the following instructions.

Title: tPA ELISA SOP

8.1.3.1. Determine the total number of samples and standards that will be assayed.
8.1.3.2. Determine the final volume of diluted antibody conjugate by multiplying the total number of samples and standards by 55µl.
8.1.3.3. Determine the volume of 100X Detection Antibody Conjugate to use by dividing the final volume of diluted antibody conjugate by 100.
8.1.3.4. Determine the volume of PET buffer to use by subtracting the volume of 100X Detection Antibody Conjugate from the final volume of dilute antibody conjugate.
8.1.3.5. Place the volume of PET buffer needed into a test tube.
8.1.3.6. Add the volume of 100X Detection Antibody Conjugate to the PET buffer. Be sure to dispense the 100X Detection Antibody Conjugate below the surface of the PET buffer and to rinse the inside of the pipet tip by pipetting up and down several times with the tip below the surface of the PET buffer.
8.1.3.7. Close the top of the test tube securely and invert several times to mix.
8.1.3.8. Label the test tube as: Diluted Antibody Conjugate in PET buffer for tPA ELISA, Store: 2-8°C, Dispose: Drain, [Date], [Initials].

8.1.4. **tPA Antigen standard:**

8.1.4.1. Aseptically add 0.5mL of ultrapure water to a vile containing tPA Plasma Standard (30ng/mL).
8.1.4.2. Aseptically add 0.5 mL of ultrapure water to a vile containing tPA Depleted Plasma Standard (0ng/mL).
8.1.4.3. Agitate both vials gently for 5 minutes.
8.1.4.4. Mix the tPA Plasma Standard and the tPA Depleted Plasma Standard in microfuge tubes according to the table below.

Concentration ng/mL	tPA Plasma Standard µL	tPA depleted plasma µL
0	0	30
10	10	20
20	20	10
30	30	0

8.1.4.5. Label the tubes as: tPA standard for ELISA, [Concentration], Store: -20°C, Dispose: Drain, [Date], [Initials].

8.1.5. **CHO samples:**

8.1.5.1. If not done already, centrifuge the samples to remove cells from the media and remove the supernatant to a new tube. Use the supernatant in the assay. This step is not necessary if the cells were removed while performing a previous SOP or if the sample has been eluted from a chromatography column.

Title: tPA ELISA SOP

8.1.5.2. In a new microfuge tube, dilute the samples 1:10 by combining 10µl of sample with 90µl PET buffer.

8.2. **Assay**

8.2.1. Equilibrate all reagents to room temperature before use.

8.2.2. Obtain the number of wells needed for the total number of standards and samples that will be assayed.

8.2.3. Reconstitute wells by adding 50µL ± 1µL of PET buffer to each well. Cover the wells with a laboratory film such as Parafilm, and agitate gently for 3-5 minutes at room temperature.

8.2.4. Add 20µL ± 1µL of each tPA standard and sample to individual wells. Mix gently with the pipettor by aspirating and dispensing 3 times.

8.2.5. Record positions of the standards and samples.

8.2.6. Cover the ELISA wells with laboratory film, and incubate for approximately 1 hour at room temperature while agitating gently.

8.2.7. Add 50µL ± 1µL of the diluted antibody conjugate to the wells. Mix gently with the pipettor by aspirating and dispensing 3 times.

8.2.8. Cover the ELISA wells with a clean sheet of laboratory film and incubate for 15 minutes at room temperature while agitating gently. This will label the bound tPA.

8.2.9. Discard the contents of the wells by turning the wells upside down on a laboratory towel and tapping several times.

8.2.10. Wash the wells four times with PET buffer as follows:

8.2.10.1. Fill each well with approximately 200µl of PET buffer.

8.2.10.2. Turn the wells upside down onto a laboratory towel and tap several times to remove the PET buffer.

8.2.11. After the fourth wash it is VERY important to tap the wells dry until all visible fluid is removed from the wells.

8.2.12. Add 100µL± 1µL TMB substrate solution to each well. Tap the wells gently (right side up) to ensure that the TMB solution settles at the bottom of the wells.

8.2.13. Cove the wells with a clean sheet of laboratory film and incubate at room temperature for 15-60minutes while agitating gently.

8.2.14. Measure the absorbance at 630nm per plate reader SOP.

8.3. **Generate a Standard Curve and Calculate Results**

Note: For detailed directions on how to generate a standard curve using Microsoft's Excel 2007 see section 9 (Attachments). Other appropriate software programs may be used to generate the standard curve.

8.3.1. Plot absorbance at 630nm against the 0, 10, 20, and 30 ng/mL standards.

8.3.2. Fit a linear trendline through the points.

8.3.3. Include the R-squared value and linear equation on the graph.

8.3.4. Use the equation to calculate the concentration of tPA in the wells.

8.3.5. Calculate the concentration of tPA in the culture media by multiplying by the dilution factor of 10.

9. Attachments:

9.1. Figure 1: Standards dilution table located in section 8.2.6.

Title: tPA ELISA SOP

9.2. Directions for generating a standard curve using Microsoft's Excel 2007

9.2.1. Open a new spread sheet in Excel 2007.

9.2.2. Enter the concentration data (X axis) from top to bottom into a column starting with 0 and ending with the number value of the highest concentration.

9.2.3. Enter the corresponding abosorbance value (Y axis) generated by the plate reader in the column directly to the right of the column used to enter the concentration data. For example:

X-value	Y-value
ng/mL	Absorbance
0	0
10	0.092
20	0.179
30	0.320

Note: Enter as many standard concentration values as were used in the assay.

9.2.4. Highlight the cells containing number values only.

9.2.5. Click on the "Insert" tab.

9.2.6. Click on "Scatter" in the "Charts" section.

9.2.7. Choose the chart-type at the top of the left column called "Scatter with only Markers" when you mouse over the choice. The chart will appear.

9.2.8. Right click on one of the data points and choose "Add Trendline…". The "Trendline Options" dialog box will appear.

9.2.9. Select "Linear" and check off "Display Equation on chart" and "Display R-squared value on Chart".

9.2.10. Click on "Close". The line, equation and R-squared value will appear in the chart.

9.2.11. To calculate the concentration of tPA in the sample, substitute the absorbance value for "y" in the equation and solve for "x" and multiply by the dilution factor of 10.

10. History:

Name	Date	Amendment
Sonia Wallman	1997	Initial release
Deb Audino	07Jul05	Put into 2005 SOP format. Added a 1:5 dilution of samples. Changed substrate to TMB
Deb Audino	04Nov05	Changed dilution process for the conjugate because the kit was changed. Increased the dilution of the samples to 1:10 from 1:5
Deb Audino	04Apr08	College name change , format history update
Kari Britt	09Jun09	Added directions for making a standard curve using Microsoft Excel 2007.

Title: tPA Activity Assay SOP

Approvals:
Preparer:_____Deb Audino_____ Date:_____03Apr08_____
Reviewer:_____Bob O'Brien_____ Date: _____03Apr08_____

1. **Purpose:**
   1.1. To measure tPA activity.
2. **Scope:**
   2.1. To measure tPA activity from cultured cells or purified fractions using the Spectrozyme substrate.
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 informed the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. pH meter SOP
   4.2. incubator SOP
   4.3. plate reader SOP (optional)
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
   7.1. pH meter
   7.2. 37ºC incubator
   7.3. plate reader with 450nm filter (optional)
   7.4. 250mL flask
   7.5. 100mL graduated cylinder
   7.6. 125mL bottle
   7.7. 1 and 10mL pipets and pump
   7.8. 10mL tube
   7.9. 1X Tris-Imidazole buffer with pH 8.4
      7.9.1. 4.04 g tris-base
      7.9.2. 2.27g imidazole
      7.9.3. 13.65g NaCl (sodium chloride)
   7.10. microtiter strips
   7.11. laboratory film such as Parafilm
   7.12. 10µM SPECTROZYME® substrate from American Diagnostica (Catalog number: 444)
   7.13. 10µL (0.04 µg/µL) tPA
   7.14. 1N HCl (or any hydrochloric acid solution for adjusting pH)
8. **Procedure:**
   8.1. **Solution Preparation**
      8.1.1. **10X Tris-Imidazole, pH 8.4.**
         8.1.1.1. Weigh and combine the following chemicals in a 250mL flask:
            4.04g tris-base

Title: tPA Activity Assay SOP

2.27g imidazole
13.65g NaCl (sodium chloride)

8.1.1.2. Add 94mL deionized water.
8.1.1.3. Add 1N HCl (Hydrochloric acid) drop-wise until pH of solution is 8.4.
Note: 1N HCl is recommended, but it is acceptable to use any hydrochloric acid solution approved by the instructor for pH adjustment.
8.1.1.4. Transfer to a 100mL graduated cylinder.
8.1.1.5. Bring volume up to 100mL with deionized water.
8.1.1.6. Transfer to a 125mL bottle.
8.1.1.7. Store at room temperature.

8.1.2. **1X Tris-Imidazole , pH 8.4**
8.1.2.1. Using 1mL and 10mL pipets, combine 1mL of 10X Tris-Imidazole, pH 8.4 and 9mL distilled water in a 10mL tube.
8.1.2.2. Store at room temperature.

8.1.3. **Spectrozyme (5nmol/mL)**
8.1.3.1. Add 2mL deionized water to lyolphilized 10 µMoles SPECTROZYME®.
8.1.3.2. Be sure that the powder is fully dissolved by inverting the bottle several times.
8.1.3.3. Storage: Reconstituted substrate may be stored for 1 week at room temperature, 2 months at 2-8ºC, or up to 6 months at –20ºC (Aliquot and freeze. Do not submit to freeze-thaw cycles).

8.2. **Assay**
8.2.1. Add 80 µL 1X Tris-Imidazole to each well
8.2.2. Add 20µL of 5nmol/mL SPECTROZYME® substrate to each well.
8.2.3. Prepare control wells.
8.2.3.1. Positive (+) Control Well: add 10 µL tPA (0.04 µg/µL) into positive (+) well.
8.2.3.2. Negative (-1) Control Well: add 10 µL Tris-Imidazole buffer into negative (-1) well.
8.2.3.3. Negative (-2) Control Well (only if testing a culture sample): add 10µl media without cells into negative (-2) well.
8.2.4. Prepare sample wells.
8.2.4.1. Place 10µL of each sample into their specified well.
8.2.5. Gently shake microtiter strip well to mix reagents.
8.2.6. Cover the strip with laboratory film.
8.2.7. Incubate wells in 37°C incubator for 1-24 hours.
8.2.8. Remove from incubator and observe tubes. Positive tubes should turn yellow while negative tubes should stay clear.
8.2.9. Optional: Read wells in a plate reader set at 450nm.

9. Attachments: N/A

Title: tPA Activity Assay SOP

10. History:

Name	Date	Amendment
Christopher Cotter Amanda Marshall	01 Jul04	Initial release
Deb Audino	01Jun05	Put into 2005 SOP format and reduced volume of assay.
Deb Audino	04Nov05	Added catalog number of Spectrozyme. Edited adding reagents to wells to clarify the section
Deb Audino	04Apr08	Updated history format. College name change

Title: Bio-Tek Elx808UI Automated Microplate Reader Standalone SOP

Approvals:

Preparer:___Ellery Raitt_____ Date ____28Feb05_____
Reviewer:__Deb Audino_____ Date ____28Feb05_____
Reviewer:__Sonia Wallman_____ Date ____28Feb05_____

1. **Purpose:**
   1.1. Operation of the Bio-Tek Elx808UI Automated Microplate Reader.
2. **Scope:**
   2.1. Applies to the Bio-Tek Elx808UI Automated Microplate Reader for performing optical density testing on solutions.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensre 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. Bio-Tek Automated Plate Reader Operators Manual
5. **Definitions:**N/A
6. **Precautions:**N/A
7. **Materials:**
   7.1. samples, standards and controls to be tested
   7.2. micropipettor.
   7.3. 96-well microplate (U, V, or flat-bottom wells are acceptable).
8. **Procedure:**
   8.1. **Preparation**
      8.1.1. Assemble samples to be tested . A minimum of 100µL for each well is required.
      8.1.2. Load samples into microplate starting at the top left corner (location A1). Load proceeding samples down the microplate, B1, C1, D1, etc. Refer to Figure 1 for a map of the microplate.
   8.2. **Operation**
      8.2.1. Turn the power switch to the ON position (located on the rear of the right side panel). The equipment will perform a system self-test to verify that the components are operating properly and that the internal software has not been corrupted (less than one minute).
      8.2.2. Press the **READ** key on the bottom right corner of the control pad or press the corresponding softkey for **READ** below the LCD display. See Figure 2.
      8.2.3. Type in "01" to select assay number 01, Quick Read assay (if not already selected). Press **Enter** to continue.
      8.2.4. Select either **Single** or **Dual** wavelength and press Enter.
      Note: If Dual wavelength is selected, the previous wavelength setting for Single will be used for the measuring wavelength. The second wavelength will be the reference wavelength.

Title: Bio-Tek Elx808UI Automated Microplate Reader Standalone SOP

8.2.5. Press the softkey corresponding to the wavelength to be used. Press **Enter.**
8.2.6. Type in the number of samples to be tested. The samples will be measured starting with well A1, then B1, C1, etc. If more than 8 samples were loaded, the machine will automatically move to column 2 and begin measuring at well A2.
8.2.7. Press **Enter** to continue.
8.2.8. Open the lid to the carrier and load microplate into reader. Well A1 must be located in the top left corner. See Figure 3.
8.2.9. Close the cover and press the **READ** key on the control pad.
8.2.10. When the reader has completed the measurements, data will automatically be sent to printer.
8.2.11. When finished, turn the power off.

9. Attachments:

9.1. Figure 1: Microplate Map
9.2. Figure 2: Control Pad
9.3. Figure 3: Loading a Microplate

10. History:

Name	Date	Amendment
Ellery Raitt	03Mar05	Initial release

Title: Bio-Tek Elx808UI Automated Microplate Reader Standalone SOP

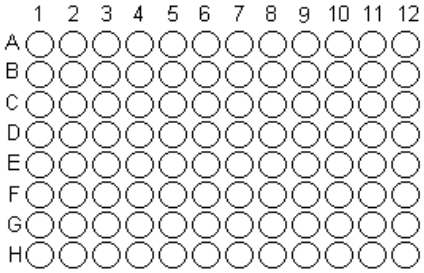


Figure 1: Microplate Map

Figure 2: Control Pad

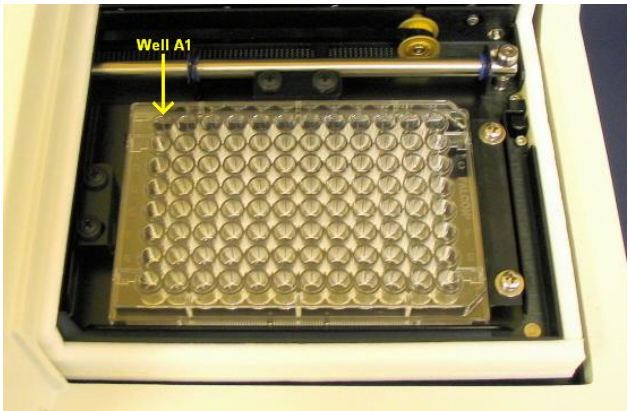


Figure 3: Loading a Microplate

Quality Control Technician (Chemistry)

REF	Key Functions & Tasks (Quality Control Chemistry Technician)
1	**Work in compliance with EH&S.**
1.a	Wear appropriate personal protective equipment.
1.b	Work in controlled environments.
1.c	Participate in emergency drills and emergency response teams.
1.d	Use routine lab safety procedures, identify unsafe conditions, and take corrective action.
1.e	Appropriately and safely access production and laboratory equipment.
1.f	Handle, label, and dispose of hazardous / biohazard materials.
1.g	Access and utilize MSDS.
1.h	Perform permitting procedures.
1.i	Carries out operations with attention to OSHA and EPA regulations, and other applicable state and federal regulations.
1.j	Participate in all company safety training and audits as required.
1.k	Assists with waste treatment operations.
1.l	Dispose of wastes .
1.m	Follows SOPs, written test procedures, safety, regulatory requirements, and approved license requirements.
1.n	Use hoods where appropriate.
1.o	Dispose of sharps properly.
2	**Work in compliance with cGMPs.**
2.a	Follow SOPs for all operations.
2.b	Records laboratory data and completes lab records as required.
2.c	Maintain equipment and instrument logbooks.
2.d	Control and receipt of raw materials.
2.e	Maintain training documentation.
2.f	Maintain equipment and methods in a validated state.
2.g	Working in controlled/classified areas (gowning, aseptic technique).
2.h	Ensure appropriate flow of personnel, equipment, and materials.
2.i	Change control for process, equipment, and documentation.
2.j	Label and apply status to equipment and materials.
2.k	Identify and report exception events and CAPA.
2.l	Review data.
2.m	Participate in change control activities.
2.n	Archive documentation/data.
3	**Clean and maintain production areas.**
3.a	Housekeeping / pest control.
3.b	Sanitize and clean of controlled spaces.
3.c	Preparation of cleaning materials and solutions
3.d	Document cleaning.
4	**Maintain effective communication.**
4.a	Maintain security and confidentiality.
4.b	Respond appropriately to internal auditors and external inspectors.
4.c	Assist in writing, reviewing, and commenting on technical documents.
4.d	Consult appropriately with internal customers.
4.e	Report result to appropriate departments.
4.f	Interact with vendors.

REF	Key Functions & Tasks (Quality Control Chemistry Technician)
5	**Receive and/or collect samples.**
5.a	Receive/collect samples (stability, in-process, water, raw materials, final product, environment, validation) per batch records or plans.
5.b	Monitor controlled equipment.
5.c	Maintain equipment logs.
5.d	Label samples appropriately.
5.e	Record sample collection and distribution (storage and chain of custody).
6	**Laboratory Work.**
6.a	Prepare reagents.
6.b	Prepare samples.
6.c	Review testing procedures
6.d	Execute testing.
6.e	Perform mathematical and statistical calculations as appropriate.
6.f	Evaluate data with regard to specification.
6.g	Perform scheduled sanitizations of hoods.
6.h	Prepare and standardize probes and ancillary instruments.
6.i	Visually inspect equipment.
6.j	Maintain equipment logs and status tags.
6.k	Complete, review and approve equipment process records.
7	**Perform Chemical and biochemical testing.**
7.a	Perform chemical and biochemical testing for identity, potency, purity, consistency, and stability.
7.b	Perform chemical and biochemical testing for raw material, water, and other environmental and validation samples.
8	**Managing Information**
8.a	Support data trending activities.
8.b	Utilize Laboratory Information Management Systems (LIMS)
8.c	Records observations, generates reports, and maintains accurate records
9	**Provide Technical and/or Validation Support**
9.a	Troubleshoots basic technical issues and investigations of OOS results, instrument malfunctions,and methodology problems.
9.b	Assist in executing validation procedures
10	**Maintain laboratory systems and equipment**
10.a	Perform preventive maintenance (PM).
10.b	Standardize laboratory instrumentation.
10.c	Identify and assist in troubleshooting instrument and equipment failures.

Short Protocol for Human Tissue Plasminogen Activator (tPA) Production in a Spinner Flask

By Susan Clark and Kari Britt

This protocol can be used to culture Chinese Hampster Ovary (CHO) cells in a spinner flask for Tissue Plasminogen Activator (tPA) production. This is an alternative protocol to growing the cells in a bioreactor (See: Batch Culture of Recombinant tPA Secreting CHO Cells (ATCC CRL9606) SOP). This protocol requires two spinner flasks, a 100mL and a 500mL for scaling up the production. This protocol is intended for use with the ATCC CRL 9606 CHO cell line. The cells are cultured for 5-7 days in a 100mL spinner flask and then for 5-7 days in a 500mL spinner flask.

Media Preparation

90% Ham's F-12, 10% Fetal Bovine Serum (1% Gentamycin is optional)

1. Clean, assemble and autoclave a 100mL spinner flask.
2. Aseptically combine 90mL of Ham's F-12 and 10mLof Fetal Bovine Serum. 1mL of 10mg/mL gentamycin can also be added to this solution to help prevent contamination. Antibiotic addition is highly recommended if a biological safety cabinet is not available.
3. Filter sterilize the media.
4. Aseptically transfer the media to the 100mL spinner flask.
5. Proof the media for contamination by incubating at 37°C, 5% CO_2 while spinning gently for at least 24 hours.
6. Visually observe the media for contamination. Dispose if contaminated. If not contaminated, store the media at 2-8°C until needed or proceed to inoculation.

Inoculation

1. If the media has been refrigerated, pre-warm it in the 37°C incubator until it seems like the media is approximately 37°. If the media is already warm, proceed to step 2.
2. Thaw a cryovial of ATCC CRL9606 CHO cells in a 37°C waterbath.
3. Aseptically transfer the contents of the cryovials to the 100mL spinner flask.
4. Gentle swirl the flask to mix.
5. Return the 100mL spinner flask to the incubator and incubate at 37°C, 5% CO_2 while spinning gently.
6. Immediately take a timepoint sample.

Sampling

It is recommended to take a timepoint sample every 24 hours.

1. Aseptically remove 1.5-2.0mL of culture. Take an optical density (OD) reading using a spectrophotometer. Determine the viable cell concentration using a trypan blue assay (See: Trypan Blue SOP). Take a pH reading. If a biolyzer is available measure analytes such as glucose and lactate.
2. Centrifuge the remaining sample at high speed for 5 minutes. Remove the supernatant to a clean test tube and store at 2-8°C until needed for ELISA, tPA activity assay or SDS PAGE.

Scale-Up

It is recommended to prepare and proof the media (Steps 1-5 below) for scale-up after the 100mL spinner flask has been incubating 3-4 days. After approximately 5-7 days the viable cell concentration should be approaching 1 x 10^6 cells/mL in the 100mL spinner flask. At this point scale-up to the 500mL spinner flask is recommended.

1. Clean, assemble and autoclave a 500mL spinner flask.
2. Aseptically combine 450mL of Ham's F-12 and 50mL of Fetal Bovine Serum. 5mL of 10mg/mL gentamycin can also be added to this solution.
3. Filter sterilize the media.
4. Aseptically transfer approximately 400mL of the media to the 500mL spinner flask. Set aside the excess media for use in cryopreservation.
5. Proof the media for contamination by incubating at 37°C, 5% CO_2 while spinning gently for at least 24 hours.
6. Visually observe the media for contamination. Dispose if contaminated. If not contaminated, store the media at 2-8°C until needed or proceed to inoculation (Steps 6 and 7 below).
7. If the media has been refrigerated, pre-warm it in the 37°C incubator until it seems like the media is approximately 37°. If the media is already warm, proceed to step 7.
8. Aseptically transfer the contents of the 100mL spinner flask to the media in the 500mL spinner flask.
9. Gentle swirl the flask to mix.
10. Return the 500mL spinner flask to the incubator and incubate at 37°C, 5% CO_2 while spinning gently.
11. Immediately take a timepoint sample, and continue to sample every 24hrs.

Cell Harvest and Cryopreservation

After approximately 5-7 days of incubating in the 500mL spinner flask, viable cell concentration should be approaching 1 x 10^6 cells/mL. At this point cell harvest is recommended.

1. For cryopreservation of the working cell bank a storage menstrum needs to be prepared. Combine 40mL of Ham's F-12, 5mL of Fetal Bovine Serum. Filter sterilize the media and then add 5mL of autoclaved glycerol. Alternatively, 45mL of excess media set aside during scale-up can be used. In this case, simply add 5mL of autoclaved glycerol to the media.
2. Aliquot the cell culture into 30 or 50mL centrifuge tubes. Centrifuge at 3000 x g for 10 minutes.
3. Remove the supernatant. The supernatant can be used for tPA purification (chromatography) if desired.
4. Resuspend the pellet with 1mL of storage menstrum and dispense into a 1.5mL cryovial. Store at -80°C.

Refer to the Batch Culture of Recombinant tPA Secreting CHO Cells (ATCC CRL9606) SOP for detailed step by step directions for cell harvest and cryopreservation. ELISA, SDS PAGE, tPA activity assay and chromatography standard operating procedures are included in this lab manual.

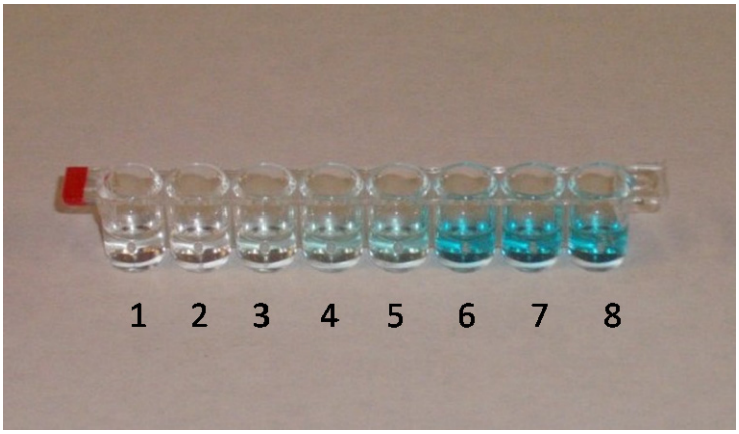


Figure 1: tPA ELISA

Well	Sample	Sample and tPA Concentration (ng/mL)
1	Blank	0
2	Standard	0
3	Standard	10
4	Standard	20
5	Standard	30
6	3rd day in 500mL flask	23.6
7	5th day in 500mL flask	26.5
8	6th day in 500mL flask	30

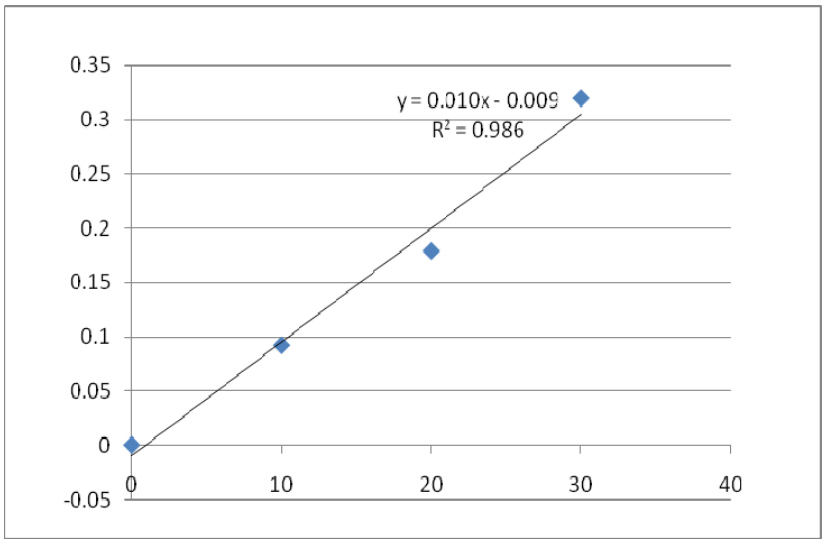


Figure 2: Standard Curve for tPA ELISA (See tPA ELISA SOP for directions to generate a standard curve).