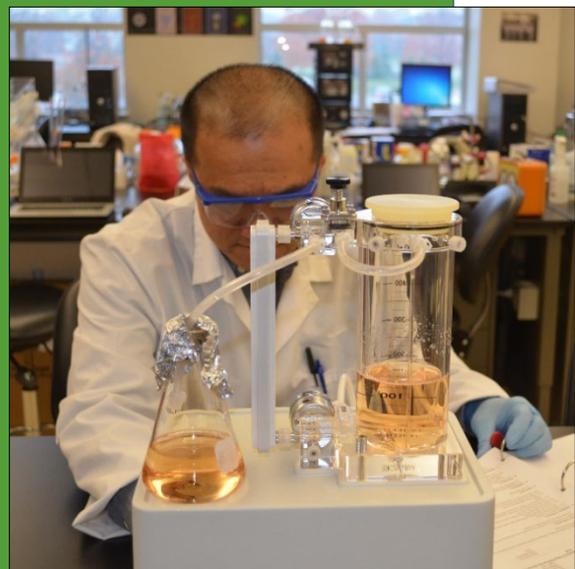




NORTHEAST BIOMANUFACTURING CENTER AND COLLABORATIVE MANUFACTURE OF MONOCLONAL ANTIBODIES LABORATORY MANUAL



NBC2

MANUFACTURE OF MONOCLONAL ANTIBODIES LABORATORY MANUAL

This lab manual was developed for a 4 Credit Biomanufacturing Laboratory/Lecture Course at Montgomery County Community College. All laboratory experiments are conducted using Standard Operation Procedures (SOPs), Batch Records, and data forms where appropriate. Included are procedures for the upstream processing, downstream processing and quality control testing used in the production of an anti IL-8 mAb from recombinant CHO DP-12 cells.

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**Upstream Processing:
CHO DP12 Growth and Production of
Anti IL-8 mAb**

Flow Chart: mAb Upstream Process

Inoculate 100ml spinner flask with $\sim 1.8 - 2.0 \times 10^7$ cells from cell bank



Monitor the cell growth every 24 hours
Perform O.D.₆₅₀, cell concentration and cell viability assays and pH measurement
Save 1ml sample of conditioned medium at each time point for performing glucose and lactate assays and API concentration by ELISA



Scale up to 1L bioreactor when the cell concentration reaches to $\sim 1.0 - 2.0 \times 10^6$ cells/ml
Usually day 6 or 7 or when cell concentration indicates that the culture has reached plateau phase



Monitor the cell growth in the bioreactor every 24 hours by performing O.D.₆₅₀, cell concentration, cell viability and pH and DO. Save 1ml sample of conditioned medium at each time point for performing glucose and lactate assays and API concentration by ELISA



Harvest the Bioreactor when the cell concentration reaches to $1.0 - 2 \times 10^6$ cells/ml.
Usually day 6 or 7 or when cell concentration indicates that the culture has reached plateau phase



Refer to mAb downstream process flow chart for harvest, centrifugation and filtration steps.

SOP: Batch Culture of Anti IL-8 Monoclonal Antibody Secreting CHO DP-12 Cells

Approvals:

Preparer: Hetal Doshi and Cianna Cooper
Reviewer: Robin Zuck
Reviewer: Dr. Maggie Bryans

Date: 20MAR20
Date: 23MAR20
Date: 24MAR20

1. Purpose:

1.1. Batch culture of the CHO DP12 cell line for the production of recombinant human Anti IL-8 monoclonal antibodies. Cells will be cultured in 100ml spinner flask and scaled up to 1L in a bioreactor.

2. Scope: Applies to the production of recombinant Anti IL-8 monoclonal antibodies from recombinant Chinese Hamster Ovary (CHO) DP12 clone.

3. Responsibilities:

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

4. References:

- 4.1. CHO DP12-ATCC® CRL-12444/12445 cell line construction culture method
<https://www.atcc.org/products/all/CRL-12445.aspx>
- 4.2. SOP: Labconco Purifier Class 2 Biological Safety Cabinet Operation, Document No. UP 1
- 4.3. SOP: Bellco Spinner Flask(100ml) Cleaning and Autoclaving, Document No. UP 2
- 4.4. SOP: Oakton PC 700 Bench Series pH/Conductivity/°C/°F Meter, Document No MET1
- 4.5. SOP: Glucose Determination Assay Using Spectrophotometer, Document No. QCB 3
- 4.6. SOP: Lactate Determination Assay Using Spectrophotometer, Document No. QCB 4
- 4.7. SOP: Trypan Blue Assay, Document No. UP6
- 4.8. SOP: Quantification of CHO DP12 Derived Anti IL-8 Monoclonal Antibody by ELISA, Document No. QCB11
- 4.9. SOP: Applikon EZ-Control Bioreactor Controller Operation, Document No. UP 4

5. Precautions:

- 5.1. Use BL2 safety measures and discard waste in biohazard containers.
- 5.2. Routine care should be exercised in the handling of buffers and samples of biological materials, which may have harmful biological activity in the case of accidental ingestion, needle stick etc.
- 5.3. Gloves, a lab coat and protective eyewear should be worn when handling buffers and samples.

6. Equipment and Materials:

- 6.1. Equipment
- 6.1.1. Biological safety cabinet
- 6.1.2. CO₂ incubator
- 6.1.3. Fisher Scientific Isotemp Low speed magnetic stirrer
- 6.1.4. Clean and autoclaved 100 ml Bellco spinner flasks
- 6.1.5. Thermo Scientific Biomate UV-visible recording spectrophotometer
- 6.1.6. Thermo Scientific Evolution 220 UV-vis spectrophotometer
- 6.1.7. Oakton PC 700 Bench Series pH/Conductivity/°C/°F Meter

SOP: Batch Culture of Anti IL-8 Monoclonal Antibody Secreting CHO DP-12 Cells

- 6.1.8. Fisher Scientific Isotemp 37°C water bath
- 6.1.9. Fisherbrand microcentrifuge
- 6.1.10. Nikon E100-LED Compound Light Microscope with 100X magnification (10X objective lens)
- 6.1.11. Hemocytometer with cover glass
- 6.1.12. Biorad iMark Microplate reader
- 6.1.13. Applikon EZ-control bioreactor controller with A 3-liter glass autoclave bioreactor and the processor
- 6.1.14. 500 ml and 1L liquid addition/feed bottles
- 6.1.15. 250 ml glass feed bottle for 150 ml alkaline solution
- 6.1.16. 100 ml glass bottle
- 6.1.17. Male and female autoclave connectors
- 6.1.18. Tubing clamps
- 6.1.19. Gas filters, 0.2µm
- 6.1.20. Autoclavable silicone tubing size 14(1.6mm interior diameter)
- 6.1.21. Autoclavable silicone tubing size 16(3.1mm interior diameter)
- 6.1.22. Autoclavable silicone tubing size 25(4.8mm interior diameter)
- 6.1.23. Laboratory gasses: Air compressor, CO₂, O₂(optional)
- 6.2. Materials:
 - 6.2.1. Vials of CHO DP12 cells (ATCC CRL-12445/12444)
 - 6.2.2. Dubblecco's Modified Eagle's Medium (DMEM) Corning # 10-013 CV
 - 6.2.3. Superlow IgG Fetal Bovine Serum (Hyclone # SH3089802)
 - 6.2.4. Insulin-Transferrin Selenium (ITS-G) 100X (Gibco # 41400-045)
 - 6.2.5. 0.2mM methotrexate stock solution (1000X) in PBS
 - 6.2.6. Nalagene 250 ml 0.2 µm filter units
 - 6.2.7. Trypan Blue (0.4% solution)
 - 6.2.8. 10X PBS
 - 6.2.9. 150 ml of 1M NaHCO₃ (sodium bicarbonate)
 - 6.2.10. 10mg/ml gentamycin
 - 6.2.11. O₂ Electrolyte solution for DO probe
 - 6.2.12. Control serum for glucose and lactate assay
 - 6.2.13. Glucose oxidase assay kit
 - 6.2.14. Lactate assay kit
 - 6.2.15. Glucose standard
 - 6.2.16. Lactate standard
 - 6.2.17. 100ml and 250 ml graduated cylinder
 - 6.2.18. Sterile serological pipettes (2ml, 5ml, 25 ml, and 50 ml)
 - 6.2.19. Pipette aid
 - 6.2.20. Spectrophotometer UV/Vis cuvettes and cuvette rack
 - 6.2.21. Oakton pH 4.0 and pH 7.0 standard buffers
 - 6.2.22. 50 ml beakers
 - 6.2.23. 1-T25 vented tissue culture flask for blank
 - 6.2.24. Test tube rack

SOP: Batch Culture of Anti IL-8 Monoclonal Antibody Secreting CHO DP-12 Cells

- 6.2.25. 1.5 ml microfuge tube and tube holder
- 6.2.26. P20, P200, and P1000 micropipettes and compatible tips
- 6.2.27. Sterile 250ml glass bottles for storage of CHO cell media
- 6.2.28. Aluminum foil
- 6.2.29. Autoclave tape

7. Procedure:

The batch record should be completed step by step by operator of the task and the verifier of the task.

- 7.1. Preparation of CHO DP-12 Complete Growth media- DMEM, 90% Super low IgG Fetal Bovine Serum, 10% 1X Insulin-Transferrin Selenium (ITS-G), 200nM methotrexate solution.
 - 7.1.1. Prepare biological safety cabinet per Labconco Purifier Class 2 Biological Safety cabinet (BSC) Operation SOP
 - 7.1.2. Gather the following items, spray with 70% isopropanol, and place in the biological safety cabinet.
 - 7.1.2.1. Pipette aid (wipe with paper dampened with 70% IPA)
 - 7.1.2.2. 5ml sterile pipettes
 - 7.1.2.3. 25ml sterile pipettes
 - 7.1.2.4. 500ml bottle of pre-sterilized DMEM media
 - 7.1.2.5. Super low IgG Fetal Bovine Serum
 - 7.1.2.6. 250 ml sterile 0.22 μm filter unit
 - 7.1.3. 120 ml Complete Growth media:
 - 7.1.3.1. Add 108 ml of DMEM, 12 ml of Super Low IgG FBS, 1.2 ml ITS-G (100X), and 0.120 ml methotrexate (1000X) to the top portion of the filter unit and sterile filter
- 7.2. Preparation of Spinner flask and Blank tube
 - 7.2.1. Obtain 100 ml Bellco spinner flask that has been previously cleaned and autoclaved per SOP, Bellco Spinner Flask (100ml) Cleaning and Autoclaving
 - 7.2.2. Aseptically transfer 98ml of the prepared complete growth media to the 100ml spinner flask.
 - 7.2.3. Aseptically transfer 20 ml of the prepared complete growth media to a T25 vented tissue culture flask.
 - 7.2.4. Label the spinner flask as CHO DP12, [date], [group#], [Operator initials]. Label the T25 tissue culture flask as BLANK, [date]. [group#], [Operator initials].
 - 7.2.5. Place spinner flask containing CHO cell media in the CO₂ incubator. Set the speed of the magnetic stirrer to 60 rpm to ensure an even mixing of the culture without foaming. Make sure to loosen side arm caps of spinner flask once in incubator.
 - 7.2.6. Place T25 tissue culture flask containing complete growth media in the CO₂ incubator.
 - 7.2.7. Verify that the temperature is $37 \pm 0.5^{\circ}\text{C}$ and percentage of CO₂ is $5 \pm 0.5\%$.
 - 7.2.8. Check media for contamination after a minimum of 24 hours.
- 7.3. Inoculation of Spinner flask
 - 7.3.1. Prepare biological safety cabinet per SOP.
 - 7.3.2. Wipe the pipette with tissue paper dampened with 70% isopropanol and place in the BSC.

SOP: Batch Culture of Anti IL-8 Monoclonal Antibody Secreting CHO DP-12 Cells

- 7.3.3. Remove spinner flask from the incubator after tightening the side arm caps. Spray spinner flask and place it in the prepared biological safety cabinet for inoculation.
 - 7.3.4. Remove two vials of CHO DP12 cells from storage in the -80°C freezer and record removal of the two vials in the ScienTemp -80°C freezer log. **Each vial should contain between 5×10^6 and 10×10^6 cells/vial to obtain concentration of 1.8×10^5 - 2.2×10^5 cells/ml after inoculation.**
 - 7.3.5. Thaw vial contents rapidly by agitation in a $37^\circ\text{C} \pm 0.5^\circ\text{C}$ water bath. **Hold the cryovial in the water without submerging the cap area to avoid contamination.**
 - 7.3.6. Spray vials with 70% isopropanol, and place in the biological safety cabinet.
 - 7.3.7. Aseptically transfer the entire contents of both 1 ml vials of thawed CHO DP12 cells into the Bellco spinner flask labelled CHO DP12 [date], [group#], [operator initials] using 2 ml sterile pipette. Do not add cells to the T25 tissue culture flask labelled BLANK, [date], [group#], [operator initials].
 - 7.3.8. Swirl to mix. Place the spinner flask on the low speed magnetic stirrer set at 60 rpm in the CO₂ incubator at 37°C with 5% CO₂ for 10 minutes. Make sure to loosen side arm caps of spinner flask once in incubator.
 - 7.3.9. Take day 0 samples following the procedure described in 7.4.1.
- 7.4. Monitoring/Sampling the cell culture
- After inoculation, take 3 ml samples of the culture immediately (Day 0, 10 minutes immediately after inoculation) and at specified time points to monitor cell growth and viability and culture conditions. Analyze samples from each time point using tests for: (1) optical density at 650 nm, (pH), (3) viable cell count (trypan blue assay), (4) glucose/lactate concentration and (5) anti IL-8 concentration. Samples will be tested every 24 hrs. + 2 hrs. until the cell density of $\geq 1 \times 10^6$ cells/ml (typically day 6) is reached and the culture can then be inoculated into the bioreactor.
- 7.4.1. Sampling the culture
 - 7.4.1.1. Prepare biological safety cabinet per SOP
 - 7.4.1.2. Collect the following items:
 - 5-microfuge tubes labeled “blank”, “cell count”, “trypan blue”, “microcentrifuge counterbalance” and “anti IL-8-vessel name, day of culture, group initials, date”
 - 1-microfuge tube holder
 - 2-spectrophotometers cuvettes (1 labeled “Sample” and 1 labeled “Blank”)
 - 1-cuvette holder
 - 1-P1000 pipette
 - 1-P20 pipette
 - 1 mL pipette aid
 - 7.4.1.3. Collect the following items, spray with 70% IPA and place in Biological Safety Cabinet:
 - 1-15 mL conical tube and conical tube holder
 - 1-Microfuge tube labelled blank and microfuge tube holder
 - 1-Pipette aid

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- 1-5 mL individually wrapped serological pipette
 - 1-1 mL individually wrapped serological pipette
- 7.4.1.4. Remove spinner flask, BLANK T25 tissue culture flask from CO2 incubator, spray 70% IPA and place in biological safety cabinet
- 7.4.1.5. Using aseptic technique, remove 3 mL of sample from CHO DP12 labeled Spinner Flask and place into the 15 mL conical tube
- 7.4.1.6. Using aseptic technique, remove 1 mL from BLANK, [date], [group#], [operator initials] and place into a 1.5 mL microfuge tube labeled blank
- 7.4.1.7. Return CHO DP-12 labeled Spinner Flask and BLANK T25 tissue culture flask to the CO2 incubator, making sure to loosen side arm caps of spinner flask once in incubator
- 7.4.2. Testing Culture Samples
- Collect and analyze 3 mL sample from each time point and test each sample for: (1) optical density at 650 m, (2) viable cell count (trypan blue assay), (3) pH (4) glucose and lactate concentration and (5) anti-IL-8-concentration.
- 7.4.2.1. Mix 3 mL cell suspension by inverting the 15 mL tube several times. Transfer 100 µl of cell suspension to the tube labelled “cell count”
- 7.4.2.2. Cell concentration and viability
- 7.4.2.2.1. Using the 100 µl of cell suspension from microfuge tube labelled “cell count” from the step above determine cell count and cell viability using Trypan Blue Assay SOP
- 7.4.2.2.2. **Record all data in the production batch record**
- 7.4.2.3. pH measurement
- 7.4.2.3.1. Calibrate pH Meter per Oakton PC 700 Bench Series pH/Conductivity/°C/°F Meter SOP
- 7.4.2.3.2. Using the remaining 2.9 mL sample in the 15 mL conical tube measure the pH reading per Oakton PC 700 Bench Series pH/Conductivity/°C/°F Meter SOP
- 7.4.2.3.3. Record the pH on data sheet
- 7.4.2.3.4. Rinse the pH probe with milliQ water and blot dry with kimwipes
- 7.4.2.3.5. Rinse the pH probe with 70% ethanol and blot dry with kimwipes
- 7.4.2.3.6. Rinse the pH probe with milliQ water and blot dry with kimwipes. Store the pH probe in the pH storage solution as per pH meter SOP
- 7.4.2.4. OD Measurement at 650 nm
- 7.4.2.4.1. Turn on the Biomate 5 UV-Vis spectrophotometer at least 10 minutes prior to measuring the absorbance
- 7.4.2.4.2. Select general test by pressing the key at bottom of the display screen reading “general test”
- 7.4.2.4.3. Under the general test menu select “Fixed” by moving the cursor using up/down arrow key. Press “Enter” to select fixed method
- 7.4.2.4.4. Select wavelength in the fixed method page by using up/down arrow key.
- 7.4.2.4.5. Using the numeric key enter 650 nm in the popup box. Press ENTER when finished.
- 7.4.2.4.6. Using the up/down arrow key select “NUMBERS OF SAMPLES” press ENTER.

SOP: Batch Culture of Anti IL-8 Monoclonal Antibody Secreting CHO DP-12 Cells

- 7.4.2.4.7. Using the numeric key enter 1 in the popup box. Then press ENTER.
- 7.4.2.4.8. Transfer the 1 mL Blank from microfuge tube into the cuvette labelled "Blank."
- 7.4.2.4.9. Using the same 2.9 mL of sample from step 7.4.2.1., transfer 1 mL of sample to the cuvette labelled "Sample." Pipet the sample up and down in the cuvette several times to mix.
- 7.4.2.4.10. Press RUN on the spectrophotometer. Follow the instructions displayed on the screen of the spectrophotometer. Record the reading in the data table of the production batch record.
- 7.4.2.4.11. Print the result by pressing the button below the print option displayed on the screen
- 7.4.2.4.12. Remove the sample and blank cuvettes from the spectrophotometer and discard the sample and blank after bleaching the sample with 10 % bleach in the sink.
- 7.4.2.4.13. Discard the cuvette in the biohazard waste.
- 7.4.2.5. Anti-IL-8-concentration, Glucose and Lactate Measurement
 - 7.4.2.5.1. Remove 1 mL of the remaining 1.9 mL sample and place in the 1.5 mL micro centrifuge tube labelled "cell". Centrifuge in the microcentrifuge for 5 minutes. Make sure to counterbalance the sample microfuge tube with the microcentrifuge the labeled "counterbalance" containing 1 mL of water.
 - 7.4.2.5.2. Remove the supernatant from the sample and place in the microfuge tube labeled "anti IL-8-vessel name, day of culture, group initials, date". Store at 2-8°C in a microfuge tube storage box labeled with Date, Group Name, Vessel Name for measurement of Anti IL-8, Glucose and Lactate concentration.
 - 7.4.2.5.3. Add 10% Bleach solution to the remaining cell suspension and discard in the Biohazard waste

7.5. Scale up to 1L bioreactor

Note: When the suspension culture of CHO cells reaches a concentration equal to or greater than 1×10^6 cells/mL (typically around day 6), the entire contents of the spinner flask will be added to the bioreactor containing 1L of CHO DP12 Cell Growth Media.

- 7.5.1. Prepare and autoclave addition/feed bottles
 - 7.5.1.1. Prepare 250 mL alkaline bottle containing 150 mL of 1M NaHCO₃ in deionized water
 - 7.5.1.1.1. Weigh out 12.6 ± 0.1 g of NaHCO₃ and transfer to a 250 mL beaker.
 - 7.5.1.1.2. Using a 250 mL graduated cylinder, measure 100 mL MilliQ water and add to the 12.6 ± 0.1 g of NaHCO₃ in the beaker. Add a magnetic stir bar and stir on a magnetic stirrer to dissolve.
 - 7.5.1.1.3. Transfer dissolved 1 M NaHCO₃ solution to 250 mL graduated cylinder and bring to 150 mL volume with MilliQ water.
 - 7.5.1.1.4. Label the bottle as 1M NaHCO₃, [date], [initials], [group number], storage: room temp, disposal: drain.
 - 7.5.1.1.5. Transfer 150 mL solution to the labeled 250 mL alkaline feed bottle

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- 7.5.1.1.6. Prepare labeled alkaline bottle for bioreactor - add lid and tubing per Applikon EZ-Control Bioreactor Controller Operation SOP.
- 7.5.1.2. Prepare 100 mL 1X PBS from 10X PBS stock solution
 - 7.5.1.2.1. In a 100 mL graduated cylinder measure 10 mL 10X PBS and bring to 100 mL volume with 90 mL MilliQ water
 - 7.5.1.2.2. Add 100 mL of 1X PBS to the Bioreactor vessel. (NOTE: Do not autoclave the bioreactor with the Complete Growth Media containing FBS).
- 7.5.1.3. Prepare 1L addition bottle with tubing and autoclavable male connector attached for autoclaving per Applikon EZ-Control Bioreactor Controller Operation SOP, steps 8.2.4).
- 7.5.1.4. Prepare the Applikon bioreactor with attached alkaline bottle for autoclaving per Applikon EZ-Control Bioreactor Controller Operation SOP (steps described in 8.2 and 8.3)
- 7.5.1.5. Autoclave the Applikon bioreactor containing 100 mL of 1X PBS with alkaline bottle attached. Autoclave 1L addition bottle with tubing and autoclavable male connector attached per the Applikon ez-Control Bioreactor Controller Operation SOP (step 8.3)
- 7.5.2. Prepare bioreactor for Cultivation

To prepare the bioreactor for cultivation, 1L of Cell Growth Media will be prepared and transferred aseptically via a 1L feed bottle to the bioreactor vessel. After a 24-hour media hold, the media will be inoculated with cells from the spinner flask.

 - 7.5.2.1. Preparation of 1L Cell Growth Media
 - 7.5.2.1.1. Gather the following items, spray with 70% isopropanol, and place in the biological safety cabinet:
 - 1-Pipette aid (swab with tissue papers damped with 70% IPA)
 - 1-10 mL sterile pipette
 - 2-50 mL sterile pipettes
 - 2-500 mL bottle of pre-sterilized DMEM Medium
 - 2-50 mL tube of pre-sterilized, Superlow IgG fetal bovine serum (FBS)
 - 1 mL aliquot of 1000x methotrexate stock solution
 - 10 mL bottle of Insulin-Transferrin Selenium (ITS-G) 100X
 - 10mL bottle of 10mg/mL gentamycin
 - 2 -Empty 50 mL conical tubes
 - 1-autoclaved 1 L addition bottle with tubing and autoclavable male connector attached
 - 7.5.2.1.2. Aseptically remove 100 mL from one 500 mL bottle of pre-sterilized DMEM media and place in the empty two 50 mL tubes.
 - 7.5.2.1.3. Aseptically add the remaining 400 mL and an additional 500 mL bottle of pre-sterilized DMEM media to the 1L addition bottle with tubing and autoclavable male connector attached.
 - 7.5.2.1.4. Aseptically add 100 mL of Superlow IgG FBS to the 1 L addition bottle with tubing and autoclavable male connector attached

SOP: Batch Culture of Anti IL-8 Monoclonal Antibody Secreting CHO DP-12 Cells

- 7.5.2.1.5. Aseptically add 1 mL of 1000X methotrexate
- 7.5.2.1.6. Aseptically add 10 mL of ITS-G
- 7.5.2.1.7. Aseptically add the 10 mL of 10 mg/mL gentamycin.
- 7.5.2.1.8. Be sure the cap is on tightly and remove the 1 L addition bottle with tubing and autoclavable male connector attached and bring it over to the Applikon bioreactor
- 7.5.2.2. Preparation of Applikon Bioreactor and Addition of media
 - 7.5.2.2.1. Remove the Applikon bioreactor vessel from the autoclave and prepare the bioreactor for cultivation according to steps 8.3.1 to 8.3.8 of the Applikon ez-Control Bioreactor Controller
- 7.5.2.3. Addition of media
 - 7.5.2.3.1. Carefully remove the foil from the female connector on the addition port of the Applikon bioreactor.
 - 7.5.2.3.2. Carefully remove the aluminum foil from the male connector on the 1 L addition bottle and connect the male connector to the female connector on the addition port of the bioreactor
 - 7.5.2.3.3. Remove the clamp on the female connector on the addition port of the Applikon bioreactor
 - 7.5.2.3.4. On the Applikon touch screen select Menu > Manual Control > Acid Pump On
 - 7.5.2.3.5. As the pump turns feed the tubing around it. Use care to avoid pinching fingers.
 - 7.5.2.3.6. Once all of the media has transferred into the vessel turn off the acid pump. On the Applikon touch screen select Menu > Manual Control > Acid Pump Off
 - 7.5.2.3.7. Disconnect the male connector of the addition bottle from the female connector on the addition port of the bioreactor. Bend the tubing of the addition port and reattach the clamp.
- 7.5.2.4. Turn on the CO2 tank. Set the output pressure at 10 psi. on the tank regulator
- 7.5.2.5. Connect the remaining parts of the bioreactor to the controller
- 7.5.2.6. Input the set points and limits listed in the table below per the bioreactor SOP.

Parameter	pH	Temp (°C)	Stirrer (rpm)
Set Point	7.2	37	75
Upper Limit	7.3	38	76
Lower limit	7.1	36	74

- 7.5.2.7. When all control loops are at set point begin 24 hours media hold to check for contamination. Turn on the pH, Temperature and Stirrer controller. Refer to step 8.8.9.6 of the Applikon ez-control bioreactor Controller operation SOP
- 7.5.2.8. DO probe polarization
 - 7.5.2.8.1. The DO probe should be polarized for a minimum of 6 hours. Polarize DO probe by connecting the probe to the controller. After minimum of 6 hours of polarization, set DO parameters as follows:

SOP: Batch Culture of Anti IL-8 Monoclonal Antibody Secreting CHO DP-12 Cells

Parameter	% DO
Set Point	50
Upper Limit	52
Lower Limit	48

7.5.2.9. After overnight media hold check the media for contamination

7.5.2.10. Turn on the air pump and set the pressure at 10 psi.

7.5.2.11. Calibrate the DO probe per the Applikon ez-Control Bioreactor Controller Operation SOP (step 8.3.11-8.3.12).

7.5.3. Cultivation

7.5.3.1. After 24 hrs. of media hold, check media for contamination.

7.5.3.2. Inoculate the bioreactor with the cells from the spinner flask by-following the steps in section 8.4.1. of the Applikon ez-Control Bioreactor Controller Operation SOP.

7.5.3.3. Immediately after inoculation (Day 0) and at 24 hr. intervals, sample the culture to determine OD, pH, viable cell count, cell viability. glucose concentration, lactate concentration and concentration of anti-IL-8. (See step 7.6.2 for Day 0-2 and step 7.6.4. for Day 3-End of Run). **Record the data in the production batch record.**

7.6. Bioreactor Sampling/Monitoring

As in previous steps with the spinner flask, after inoculation samples are taken of the culture in the bioreactor immediately (Day 0, 10 minutes immediately after inoculation) and at specified time points to monitor cell growth and viability and culture conditions. Samples from each time point are analyzed using tests for: (1) optical density at 650 nm (2) viable cell concentration (trypan blue assay), (3) glucose and lactate concentration and (4) anti IL-8 concentration. Samples should be taken daily until the cell density of $> 1 \times 10^6$ cells/mL (typically day 6) is reached. At this cell concentration, the conditioned media in the bioreactor is harvested. **Note: In order to obtain accurate cell concentration and OD readings for Day 0 through Day 2, 25 mL sample will be taken. 5 mL samples will be taken for the later time points (Day 3-End of Run)**

7.6.1. Sampling Procedure: Day 0-2

7.6.1.1. For each time point- label:

- 2 - Spectrophotometer cuvettes as "blank" and "sample"
- microfuge tube labelled "anti-IL-8- bioreactor- time point-initials, date"
- 1 microfuge tube labeled 'Blank'

7.6.1.2. In the BSC, aseptically transfer 1 mL of the blank solution to a microfuge tube labeled blank.

7.6.1.3. Log in to Applikon EZ Controller as operator per Applikon Operator SOP

7.6.1.4. Raise the stirrer upper limit to 150 rpm

7.6.1.5. Change the stirrer setting to 125 rpm.

7.6.1.6. Spray the head plate near the sampling tube with 70% IPA.

7.6.1.7. Remove the black clamp and set on the head plate.

7.6.1.8. Pull out the autoclavable female connector and set it next to the black clamp.

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- 7.6.1.9. Place a 25 mL pipette into the sampling tube, remove 25 mL of sample and place sample in a 50 mL conical tube
- 7.6.1.10. Put the female autoclavable connector back into the sampling tube.
- 7.6.1.11. Bend the sampling tubing and place the black clamp back on the tubing.
- 7.6.1.12. Change the stirrer setting to 75 rpm.
- 7.6.1.13. Change the stirrer upper limit back to 76 rpm
- 7.6.2. Testing Culture Samples: Day 0- Day 2
 - 7.6.2.1. OD Measurement at 650nm
 - 7.6.2.1.1. Remove 1 mL of the sample from the 50 ml conical tube and place in spectrophotometer cuvette labeled "sample"
 - 7.6.2.1.2. Aseptically transfer 1 mL of the blank solution in the BSC to the 1.5 ml Eppendorf tube labelled "Blank" and place in the spectrophotometer cuvette labelled "Blank"
 - 7.6.2.1.3. Measure OD at 650 nm as per step through described in this SOP. Return 1 mL sample back to the 50 mL conical tube. Record the OD in the table of the Batch Record.
 - 7.6.2.2. Cell Concentration and Viability —Trypan Blue Assay
 - 7.6.2.2.1. Centrifuge the 50 mL tube containing sample at 900 rpm for 5 minutes.
 - 7.6.2.2.2. Carefully remove supernatant, leaving approximately 0.2 mL behind so as not to disturb the pellet. Transfer supernatant to a new 50 mL conical tube
 - 7.6.2.2.3. Re-suspend the pellet in 0.8 mL of excess supernatant from step 7.6.2.2.2 using a 5 mL pipette
 - 7.6.2.2.4. Perform the trypan blue assay per SOP on the re-suspended pellet. Record the viable cells/mL and % Viability in the table of the Batch Record
 - 7.6.2.3. Anti- IL-8, Glucose and Lactate concentrations
 - 7.6.2.3.1. Remove 1 mL of supernatant from 50 mL conical tube in step 7.6.2.2.2 and transfer to a microfuge tube labeled “anti IL-8, [bioreactor], [time point], [initials], [date]”. Store at 2-8°C in a microfuge tube storage box labeled with Date, Group Name, Vessel Name for measurement of Anti-IL-8, Glucose and Lactate concentrations.
- 7.6.3. Sampling Procedure: Day 3-End of Run (EOR)
 - 7.6.3.1. Aseptically transfer 1 mL of the Blank solution to the microfuge tube labelled “Blank”
 - 7.6.3.2. Log in to Applikon ez Controller as operator per Applikon Operator SOP
 - 7.6.3.3. Raise the stirrer upper limit to 150 rpm.
 - 7.6.3.4. Change the stirrer setting to 125 rpm.
 - 7.6.3.5. Spray the head plate near the sampling tube with 70% IPA.
 - 7.6.3.6. Remove the black clamp and set on the head plate.
 - 7.6.3.7. Pull out the autoclavable female connector and set it next to the black clamp.
 - 7.6.3.8. Place a 10 mL pipette into the sampling tube and remove 5 mL of sample and place in a 15 mL conical tube.
 - 7.6.3.9. Put the female autoclavable connector back into the sampling tube.
 - 7.6.3.10. Bend the sampling tubing and place the black clamp

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- 7.6.3.11. Change the stirrer setting to 75 rpm
- 7.6.3.12. Change the stirrer upper limit back to 76 rpm
- 7.6.4. Testing Culture Samples Day 3- End of Run (EOR)
 - 7.6.4.1. OD measurement at 650nm
 - 7.6.4.1.1. Remove 1 mL of sample from the 15 mL conical tube containing 5 mL sample and place in spectrophotometer cuvette labeled "sample"
 - 7.6.4.1.2. Transfer 1 mL of the blank solution to the spectrophotometer cuvette labelled "Blank"
 - 7.6.4.1.3. Measure OD at 650 nm as per the steps in 7.4.2.4.1. through 7.4.2.4.12. of this SOP and record the data in the table of the production batch record
 - 7.6.4.2. Cell Concentration and Viability -Trypan Blue Assay
 - 7.6.4.2.1. Remove 0.1 mL of the sample from 15 mL conical tube containing the 5 mL sample and perform the trypan blue assay per SOP
 - 7.6.4.3. Anti IL-8, Glucose, Lactate concentration
 - 7.6.4.3.1. Transfer 1 ml of the sample from the 15 mL conical tube to a 1.5 mL microcentrifuge tube labeled sample. Centrifuge the 1.5 mL tube in the microcentrifuge for 5 minutes.
 - 7.6.4.3.2. Remove 1 mL of supernatant and transfer to microfuge tube labeled "anti IL-8- bioreactor time point- initials, date." Store at 2-8°C in a microfuge tube storage box labeled with Date, Group Name, Vessel Name for measurement of Anti IL-8, Glucose, and Lactate concentrations.
- 7.7. End of Run – Culture Harvest

When the cell culture reaches the cell density between 0.8×10^6 and 1×10^6 cells/mL (typically Day 6 of the run), the conditioned media is harvested.

 - 7.7.1. Disconnect the bioreactor from the controller by following the steps 8.6.1, 8.6.2 and 8.7.1 of the Applikon EZ-Control Bioreactor Controller Operation SOP.
 - 7.7.2. Transfer the cell suspension to the 250 mL centrifuge bottles
 - 7.7.3. Centrifuge cells in pre-chilled Sorvall centrifuge, fitted with a SLA1500 rotor, at $500 \times g$ for 5 min, 4°C
 - 7.7.4. Transfer conditioned medium (CM) from centrifuge bottles to storage bottle by pipetting the supernatant being careful not to disturb the pellet.
 - 7.7.5. Sterile filter the conditioned media using the 0.2µm filter unit. Store the filtered condition media in the appropriately labeled storage bottle after addition of protease inhibitors at 4°C for short term and at -20°C for long term. For the detail information regarding the preparation and amount of protease inhibitors refer “SOP: End of Run Anti IL-8 mAb Process: Harvest, Centrifugation, TFF concentration”
 - 7.7.6. Clean bioreactor following steps 8.7.2, 8.7.3, 8.7.4 and 8.7.5 of the Applikon ez-Control Bioreactor Controller Operation SOP
- 7.8. Determine Glucose, Lactate, and anti IL-8 concentrations
 - 7.8.1. Determine the glucose concentration of all the samples collected from the spinner flask and bioreactor per the Glucose Determination Assay Using Spectrophotometer SOP.
 - 7.8.2. Determine the lactate concentration of all the samples collected from the spinner flask and Bioreactor per the Lactate Determination Assay Using Spectrophotometer SOP

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7.8.3. Determine the anti IL-8 mAb concentration of all the samples collected from the spinner flask and Bioreactor using the “SOP: Quantitation of CHO DP-12 derived Anti IL-8 Monoclonal Antibody by ELISA”.

7.9. Prepare Growth Curves

7.9.1. Plot OD, pH, viable cells, glucose, lactate, and anti-IL8 concentration vs. time (use 2 y-axes). Attach growth curve to Batch Record

7.9.2. Determine growth rate and doubling time of the 100 mL spinner flask and 1L bioreactor cultures. Attach calculations to Batch Record

8. History:

Revision Number	Effective date	Preparer	Description of Change
0	20 March 17	Cianna Cooper and Hetal Doshi	Initial release
1	24 March 20	Hetal Doshi	Added steps for measuring OD at 650 nm

Batch Record: Anti IL-8 mAb Production from CHO DP-12 Cells
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.

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: DDMMYY. For July 10, 2017 use 10JUL17.

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: Anti IL-8 mAb Production from CHO DP-12 Cells
Lot Number _____

1. Initial Media Preparation and Media hold		
1.1 Clean, assemble and autoclave one 100 mL Bellco Spinner flask per SOP.	Operator/Date	Verifier/Date
1.2. Obtain sterile 50 mL conical tube.	Operator/Date	Verifier/Date
1.3. Obtain sterile DMEM Media. Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____	Operator/Date	Verifier/Date
1.4. Obtain sterile Super Low Fetal Bovine Serum (FBS). Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____	Operator/Date	Verifier/Date
1.5. Obtain sterile 100X Insulin-Transferrin Selenium (ITS-G). Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____	Operator/Date	Verifier/Date
1.6. Obtain sterile 1000X methotrexate solution (2mM). Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____	Operator/Date	Verifier/Date
1.7. Aseptically prepare 120 mL Complete Growth Media – DMEM/10% FBS, 1X ITS-G, 200nM methotrexate. 1.7. 1 Obtain 250 mL of 0.22uM sterile filter unit. Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____ 1.7.2. Transfer the following to the top portion of the filter top unit and sterile filter: <ul style="list-style-type: none"> • 107 mL of DMEM media • 12 mL of Superlow IgG FBS • 1.12 mL of 100X ITS-G • 0.12 mL of 1000X methotrexate (2mM) 1.7.3. Label the media bottle “CHO DP12 Complete Growth Media”.	Operator/Date	Verifier/Date
1.8. Preparation for Inoculation 1.8. 1. Label spinner flask as CHO DP12, [date], [group#], [operator initials]. 1.8.2. Label 50 mL conical tube as “Blank”.	Operator/Date	Verifier/Date

Batch Record: Anti IL-8 mAb Production from CHO DP-12 Cells
Lot Number _____

1.9 Aseptically transfer 98 mL of Complete Growth Media to 100 mL spinner flask. 100mL spinner flask ID# _____ Vol of Complete Growth Media _____	Operator/Date	Verifier/Date
1.10. Aseptically transfer 20 mL of Complete Growth Media to 50 mL conical tube. 50 mL conical tube ID # _____ Vol of Complete Growth Media _____	Operator/Date	Verifier/Date
1.11.1 Place spinner flask and 50 mL tube containing CHO Complete Growth Media in the CO ₂ incubator. Make sure to loosen side arm caps of spinner flask once in incubator. 1.11.2 Set the speed of the magnetic stirrer to 60 rpm setting.	Operator/Date	Verifier/Date
1.12. Verify that CO ₂ is set to 5±0.5% and that temperature is set to 37±0.5°C. CO ₂ _____ % Temperature _____ °C	Operator/Date	Verifier/Date
1.13. Check media for contamination after a minimum of 24 hrs. Incubation start time: _____ Incubation end time: _____ Elapsed time: _____ 100mL spinner flask ID _____ Contamination: Y / N (Circle) 50 mL tube ID _____ Contamination: Y / N (Circle)	Operator/Date	Verifier/Date
Comments: 	Operator/Date	Verifier/Date

Batch Record: Anti IL-8 mAb Production from CHO DP-12 Cells
Lot Number _____

2. Inoculation of Spinner Flasks		
2.1. Remove two vials of CHO cells from storage in the -80°C freezer. Vial ID _____ Vial ID _____ Cell Concentration _____ Cell Concentration _____ Cryopreservation date _____ Cryopreservation date _____	Operator/Date	Verifier/Date
2.2. Thaw vials contents rapidly by agitation in a 37°C ± 0.5°C water bath. Water bath temperature: _____	Operator/Date	Verifier/Date
2.3. Aseptically transfer the entire contents of each 1 mL vial of thawed CHO cells into the previously prepared Spinner Flask containing 98 mL CHO Complete Growth Medium using a 1 mL sterile pipette. Swirl to mix. Do not add any CHO Cells to the 50 ml conical tube labeled “Blank”.	Operator/Date	Verifier/Date
2.4. Transfer the spinner flask and the 50 mL conical tube to the CO ₂ incubator at 37°C with 5% CO ₂ . Verify that CO ₂ is set to 5±0.5% and that temperature is set to 37±0.5°C. CO ₂ __% Temperature__ °C	Operator/Date	Verifier/Date
2.5. Place spinner flask on magnetic stirrer in the CO ₂ incubator. Make sure to loosen side arm caps of spinner flask once in incubator. Set stirrer for 60 rpm for 15 minutes ± 5 minutes and take Day 0 sample.	Operator/Date	Verifier/Date
3. Monitoring of Spinner flask Cell Culture. Immediately after inoculation of the bioreactor (Day 0) and at 1 - day intervals, sample the culture to determine OD at 650 nm, viable cell count and viability, concentration of glucose, concentration of lactate, and anti IL-8 concentration. Once the cell concentration of the cell culture reaches ≥ 1,000,000 cells/mL the cell culture is scaled up to 1 L bioreactor.		
3.1 Label 5 microfuge tubes as follows: “cells”, “cell count”, “trypan blue”, “balance”, “anti IL-8-vessel name, day of culture, group initials, , date”. 3.1.2. Label 2 spectrophotometer cuvettes as follows: “blank” and “sample” 3.1.3. Swab and take 15 mL conical tube in the BSC and label as “anti IL-8, [date], [Initials]”	Operator/Date	Verifier/Date

Batch Record: Anti IL-8 mAb Production from CHO DP-12 Cells
Lot Number _____

3.2. 1. In BSC, aseptically remove 1 mL of blank media and place in microfuge tube labelled "blank". 3.2.2. Aseptically remove 3 mL of cell suspension from spinner flask and place in labeled 15 mL conical tube.	Operator/Date	Verifier/Date
3.3. Return CHO DP-12 labeled spinner flask and blank 50 mL conical tube to the CO ₂ incubator, making sure to loosen arm caps of spinner flask.	Operator/Date	Verifier/Date
3.4.1. Remove 15 mL conical tube containing 3 mL sample conical from the BSC. 3.4.2. Remove microfuge tube containing 1 mL blank from the BSC.	Operator/Date	Verifier/Date
3.5 Cell Viability 3.5.1. Remove 100µL from 15 mL conical tube containing 3 mL sample and place in microfuge tube labeled "cells count". 3.5.2. Determine viable cell count per Trypan Blue Assay SOP. Record cell viability and concentration in the table on page 7 of the Batch Record.	Operator/Date	Verifier/Date
3.6. pH measurement 3.6.1 Calibrate pH Meter per Oakton PC 700 Bench Series pH/ Conductivity/°C/°F Meter SOP. 3.6.2. Using the remaining 2.9 mL sample in the 15 mL conical tube take the pH reading per Oakton PC 700 Bench Series pH/ Conductivity/°C/°F Meter SOP. Record pH in the table on page 7 of the Batch Record.	Operator/Date	Verifier/Date
3.7. OD 650 measurement 3.7.1. Prepare spectrophotometer per SOP. Using the same 2.9 mL sample from step 3.5, transfer 1 mL of sample to the cuvette labeled "sample". Pipet the sample up and down in the cuvette several times to mix. 3.7.2. Transfer the 1 mL of blank media from the microfuge tube labeled "blank" into cuvette labeled "blank". Measure OD reading at 650 nm per Spectrophotometer SOP. Blank the spectrophotometer using the cuvette with the blank. Record the O.D in the table on page 7 of the Batch Record.	Operator/Date	Verifier/Date

Batch Record: Anti IL-8 mAb Production from CHO DP-12 Cells
Lot Number _____

<p>3.8. Measurement of Glucose, Lactate, and Anti- IL-8 concentration 3.8.1. Remove 1 mL of the remaining 1.9 mL sample and place in the microfuge tube labelled “cells”. Place 1 mL of milliQ water in microfuge tube labeled “balance”. Centrifuge both “cells” and “balance tubes” for 5 minutes in microcentrifuge 3.8.1. Remove supernatant from the sample tube and transfer to microfuge tube labeled “anti- IL-8 vessel name (Spinner or Bioreactor), day of culture, group initials, date”. 3.8.2. Store sample at 2-8°C in microfuge storage box labeled with date, group name, for measurement of glucose, lactate, and anti-IL8 concentration. 3.8.3. Add 10% bleach solution to the remaining sample and discard in the biohazard waste.</p>	Operator/Date	Verifier/Date
<p>3.9. Repeat steps 3.1 through 3.8 every 24hrs.±2hrs. until the culture is scaled Up to 1 L Bioreactor culture. Typically, on day 6 of the inoculation.</p> <p align="right">Day 1</p> <p align="right">Day 2</p> <p align="right">Day 3</p> <p align="right">Day 4</p> <p align="right">Day 5</p> <p align="right">Day 6</p>	Operator/date	Verifier/date
<p>When the 100 mL suspension culture of CHO cells reaches a concentration of $\geq 1 \times 10^6$ cells/mL, the entire contents of the spinner flask will be added to the bioreactor containing 1L of CHO cell growth media.</p>		
<p>Comments</p>	Operator/Date	Verifier/Date

Batch Record: Anti IL-8 mAb Production from CHO DP-12 Cells
Lot Number _____

100 mL Spinner Flask ID# _____

TIME (hours)	OD 650nm	pH	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

Batch Record: Anti IL-8 mAb Production from CHO DP-12 Cells
Lot Number _____

100mL Spinner Flask ID# _____

TIME (hours)	OD 650nm	pH	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

Batch Record: Anti IL-8 mAb Production from CHO DP-12 Cells
Lot Number _____

4. Bioreactor Scale Up		
<p>4.1 Preparation of 1M NaHCO₃</p> <p>4.1.1 Label 500 mL glass feed bottle 1MNaHCO₃, [date], [initials], and storage: room temperature, disposal; drain.</p> <p>4.1.2 Weigh out 12.6. ± 0.1 grams of (NaHCO₃) sodium bicarbonate and transfer to a 250 mL beaker</p> <p>Balance ID _____ NaHCO₃ manufacturer _____ Catalog number _____ Lot number _____ Expiration Date _____</p> <p>4.1.3 Using a 250 mL graduated cylinder, measure 100mL MilliQ water and add to the NaHCO₃ in the beaker</p> <p>Volume of MilliQ water added _____ mL</p> <p>4.1.4. Add magnetic stir bar and stir on a magnetic stirrer to dissolve. Transfer dissolved NaHCO₃ to a 250 mL graduated cylinder and bring to the volume at 150 mL with MilliQ water. Transfer 150 mL 1M NaHCO₃ to labeled alkaline feed bottle</p> <p>4.1.5. Prepare labeled alkaline feed bottle for autoclave. Cover the bottle and tubing per Applikon Bioreactor Controller SOP.</p>	Operator/Date	Verifier/Date
<p>4.2. Preparation of 1X PBS</p> <p>4.2.1 In a 100 mL graduated cylinder, add 10 mL 10X PBS and bring to volume with 90 mL MilliQ water</p> <p>10X PBS: Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____ Volume of 10X PBS added: _____ mL Volume of water added: _____ mL</p> <p>4.2.2. Store the prepared 1X PBS in a bottle labelled 1X PBS, [date], [initials].</p>		

Batch Record: Anti IL-8 mAb Production from CHO DP-12 Cells
Lot Number _____

5. Prepare the controller as per the Applikon EZ- Control Bioreactor Controller Operation SOP step 8.1	Operator/Date	Verifier/Date
6. Assemble/<u>Autoclave Bioreactor</u>		
6.1. Assemble the Vessel stand if not assembled	Operator/Date	Verifier/Date
6.2. 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.) Head plate O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
6.3 Assemble Head plate Underside		
6.3.1. 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
6.3.2. Attach sample tube, sparger and thermowell. Verify that the sparger tube is aligned beneath the stirrer impeller.	Operator/Date	Verifier/Date
6.3.3 Add 100 mL of 1X PBS to the bioreactor.	Operator/Date	Verifier/Date
6.4. Attach head plate to Vessel Stand.		
6.4.1. Place the head plate onto the vessel stand, positioning the holes on the outer edge of the head plate with the bolts on the vessel stand. Secure the head plate with the 5mm fasteners.	Operator/Date	Verifier/Date

Batch Record: Anti IL-8 mAb Production from CHO DP-12 Cells
Lot Number _____

6.5 Assemble Head plate – Topside		
<p>6.5.1. Inspect the integrity of the O-ring in the condenser port of the head plate. Replace if worn or cracked. Condenser port O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.)</p> <p>6.5.2. Inspect the black seal at the bottom of the condenser underneath the retainer nut. Replace if worn or cracked.</p> <p>6.5.3. Attach the condenser to the head plate by placing the condenser into the condenser port making sure that the barbed connectors are facing out.</p>	Operator/Date	Verifier/Date
6.6. DO Probe Preparation		
<p>6.6.1. Remove the protective cap from the bottom of the stainless-steel DO probe.</p> <p>6.6.2. Inspect the screen at the bottom of the probe tip. Replace if damaged.</p> <p>6.6.3. Unscrew the membrane module from the bottom housing of the probe tip by holding the probe in the vertical position. 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)</p> <p>6.6.4. Replenish DO electrolyte. There should be 1 mL of O₂ electrolyte solution in the membrane module. Reattach the membrane module.</p> <p>6.6.5. 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)</p> <p>6.6.6. 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)</p> <p>6.6.7. Attach DO probe to the head plate.</p>	Operator/Date	Verifier/Date

Batch Record: Anti IL-8 mAb Production from CHO DP-12 Cells
Lot Number _____

6.7. Calibrate the pH probe per Applikon Bioreactor Controller Operation SOP. (Refer step 8.2.2.)		
6.7.1. Obtain pH 7 and pH 4 calibration buffers. pH 7 Buffer Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____ pH 4 Buffer Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____	Operator/Date	Verifier/Date
6.7.2. Perform 2-point calibration per Applikon EZ-control Bioreactor Controller Operation SOP (Refer step 8.2.2). 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
6.7.3 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
6.7.4. 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
6.7.5. Attach pH probe to the head plate.	Operator/Date	Verifier/Date

Batch Record: Anti IL-8 mAb Production from CHO DP-12 Cells
Lot Number _____

6.8. Preparation of liquid addition bottles and attaching the filters and tubing per the Applikon Bioreactor Controller Operation SOP.		
6.8.1 Verify that the liquid addition bottles are prepared as per the 8.2.4 section of the Applikon Bioreactor Controller Operation SOP for preparing the bioreactor.	Operator/Date	Verifier/Date
6.8.2. Mount the connections to the bioreactor by following steps listed in the section 8.2.5 in the Applikon Bioreactor Controller Operation SOP. 6.8.3. Verify all the steps are followed as per the Applikon Bioreactor Controller Operation SOP for preparing the bioreactor.	Operator/Date	Verifier/Date
6.8.4. Verify that the gas filters are open to avoid pressure difference during autoclaving.	Operator/Date	Verifier/Date
6.8.5. Verify all tubing (near the head plate) except the condenser top outlet is clamped. The condenser top outlet must remain unclamped to release pressure during autoclaving.	Operator/Date	Verifier/Date
6.8.6. Cover the tubing and the head plate with aluminum foil. Place the autoclave indicator on the aluminum foil.	Operator/Date	Verifier/Date
6.9. Autoclave the bioreactor, alkaline addition bottle and liquid addition bottles as per section 8.2.6. Applikon Bioreactor Controller Operation SOP. Autoclave at 121°C for 20 minutes, using slow exhaust per Autoclave SOP. CAUTION: Always use slow exhaust when autoclaving.	Operator/Date	Verifier/Date
6.10 Remove the Bioreactor vessel, Alkaline Bottle, Media addition bottle, Inoculum addition bottle from the autoclave.	Operator/Date	Verifier/Date
7. Preparation of Cell Growth Media		
7.1.1. Obtain two sterile 500 mL bottles of DMEM Media. DMEM Bottle 1: Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____ DMEM Bottle 2: Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____	Operator/Date	Verifier/Date
7.1.2. Obtain sterile Superlow IgG Fetal Bovine Serum (FBS). Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____	Operator/Date	Verifier/Date

Batch Record: Anti IL-8 mAb Production from CHO DP-12 Cells
Lot Number _____

7.1.3. Obtain sterile 100X Insulin-Transferrin Selenium (ITS). Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____	Operator/Date	Verifier/Date
7.1.4. Obtain sterile 1000X (0.2mM) methotrexate solution. Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____	Operator/Date	Verifier/Date
7.1.5. Obtain sterile 10 mg/mL gentamicin. Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____	Operator/Date	Verifier/Date
7.2. Place the autoclaved 1 L media addition bottle in the Biological Safety Cabinet after swabbing it with 70% Ethanol. Do Not Remove the aluminum foil from the tubing and the filter.	Operator/Date	Verifier/Date
7.3. Place the media components 7.1.1, 7.1.2, 7.1.3, 7.1.4, 7.1.5 in the Biological Safety Cabinet after swabbing with 70% Ethanol.	Operator/Date	Verifier/Date
7.4.1. Aseptically remove 100 mL from one 500 mL bottle of pre-sterilized DMEM media and place in the empty two 50 ml conical tubes. 7.4.2. Aseptically add the remaining 400 mL and an additional 500 mL bottle of pre-sterilized DMEM media to the 1L addition bottle with tubing and autoclavable male connector attached. 7.4.3. Aseptically transfer the remaining media from each 500 mL bottle to the 1L addition bottle. 7.4.4. Aseptically add 100 mL of Superlow IgG FBS to the media in the addition bottle. 7.4.5. Aseptically add 10 mL of 100X ITS-G to the addition bottle. 7.4.6. Aseptically add 1 mL of 1000X (0.2mM) methotrexate to the bottle. 7.4.7. Aseptically add 10 mL of 10 mg/mL gentamicin to the bottle.	Operator/Date	Verifier/Date
8. Connecting the bioreactor to the controller and preparing for the run. Refer to the steps 8.3.1 to 8.3.8 of the Applikon Bioreactor Controller Operation SOP.	Operator/Date	Verifier/Date
8.1 Verify that all control loops are switched off. Refer to the steps in section 8.3.1 of the Applikon EZ-Control Bioreactor Controller Operation SOP.	Operator/Date	Verifier/Date
8.2. Transfer Cell Growth Media to Bioreactor. 8.2.1. Add 1L Cell Growth Media from 1L feed bottle to the bioreactor per steps 8.3.2.4. to 8.3.2.11 of the of the Applikon Bioreactor Controller Operation SOP.	Operator/Date	Verifier/Date
8.3 Connect the sensors to the controller per step 8.3.3 of the Applikon EZ-Control Bioreactor Controller Operation SOP.	Operator/Date	Verifier/Date

Batch Record: Anti IL-8 mAb Production from CHO DP-12 Cells
Lot Number _____

8.4. Verify that deionized H ₂ O has been added to the thermowell with the Pt- 100 temperature probe. Add more deionized H ₂ O if necessary.	Operator/Date	Verifier/Date																
8.5. Verify that thermal blanket is wrapped around the vessel and plugged into the ADI 1025 unit.	Operator/Date	Verifier/Date																
8.6. Connect the stirrer motor by referring to the steps 8.3.7.1 to 8.3.7.5. of the Applikon EZ-control Bioreactor Controller Operation SOP.	Operator/Date	Verifier/Date																
8.7. Connect the alkaline bottle. Refer to the steps 8.3.8.1 to 8.3.8.9 of the Applikon EZ-Control Bioreactor Controller Operation SOP.	Operator/Date	Verifier/Date																
8.8. Input the following limits per the process SOP. <table border="1" style="width:100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: left;">Parameter</th> <th style="text-align: center;">Upper limit</th> <th style="text-align: center;">Set Point</th> <th style="text-align: center;">Lower limit</th> </tr> </thead> <tbody> <tr> <td>pH</td> <td style="text-align: center;">7.3</td> <td style="text-align: center;">7.2</td> <td style="text-align: center;">7.1</td> </tr> <tr> <td>Temperature</td> <td style="text-align: center;">38</td> <td style="text-align: center;">37</td> <td style="text-align: center;">36</td> </tr> <tr> <td>Stirrer RPM</td> <td style="text-align: center;">76</td> <td style="text-align: center;">75</td> <td style="text-align: center;">74</td> </tr> </tbody> </table>	Parameter	Upper limit	Set Point	Lower limit	pH	7.3	7.2	7.1	Temperature	38	37	36	Stirrer RPM	76	75	74	Operator/Date	Verifier/Date
Parameter	Upper limit	Set Point	Lower limit															
pH	7.3	7.2	7.1															
Temperature	38	37	36															
Stirrer RPM	76	75	74															
8.9. Turn on CO ₂ supply at regulator to the bioreactor. Tank pressure _____ Tank Volume__	Operator/Date	Verifier/Date																
8.10 Start pH, Temperature and Stirrer control loop per step 8.3.10 of the Applikon ez-Control Bioreactor Controller Operation SOP.	Operator/Date	Verifier/Date																
9. Media Hold and DO probe Polarization Perform Media hold and DO probe polarization simultaneously. Media should be held for 24 hrs. to check for contamination and DO probe should be polarized for at																		
9.1 Media Hold Verify that all control loops are at set point.	Operator/Date	Verifier/Date																
9.2. DO probe Polarization 9.2.1. Connect DO probe to the controller.	Operator/Date	Verifier/Date																
9.2.2. Check media for contamination after a minimum of 24 hrs. Incubation start time: _____ Incubation end time: _____ Elapsed time: _____ Contamination? Yes / No (Circle one.)	Operator/Date	Verifier/Date																

Batch Record: Anti IL-8 mAb Production from CHO DP-12 Cells
Lot Number _____

9.3. Start air compressor and set pressure at 10 psi.	Operator/Date	Verifier/Date								
9.4. Calibrate the DO probe per the Applikon EZ-Control Bioreactor Controller Operation SOP (step 8.3.11-8.3.12). Note: Allow DO probe to polarize for at least 6 hours before performing calibration. Record slope: Slope _____ Temperature _____	Operator/Date	Verifier/Date								
9.4.1 Set DO parameters as follows: <table border="0" style="width: 100%;"> <tr> <td style="width: 60%;"><u>Parameter</u></td> <td style="text-align: center;"><u>%DO</u></td> </tr> <tr> <td>Set point</td> <td style="text-align: center;">50</td> </tr> <tr> <td>Upper Limit</td> <td style="text-align: center;">52</td> </tr> <tr> <td>Lower Limit</td> <td style="text-align: center;">48</td> </tr> </table>	<u>Parameter</u>	<u>%DO</u>	Set point	50	Upper Limit	52	Lower Limit	48	Operator/Date	Verifier/Date
<u>Parameter</u>	<u>%DO</u>									
Set point	50									
Upper Limit	52									
Lower Limit	48									
9.4.2. Verify that slope is within expected values: 1.5-3.0 at 37°C or 2.0-4.0 at 25°C	Operator/Date	Verifier/Date								
10. Inoculation of bioreactor with 100 ml spinner flask cell suspension Inoculate bioreactor when the cell suspension of CHO cells in the spinner reaches a concentration of $\geq 1 \times 10^6$ cells/ml. Refer to step 8.4.1.1 to 8.4.1.11 of the Applikon ez-Control Bioreactor Controller Operation SOP.										
10.1 Obtain, clean autoclaved 500 mL feed bottle.	Operator/Date	Verifier/Date								
10.2. Aseptically transfer CHO DP12 cell suspension from spinner flask to 500 mL feed bottle per Batch CULTURE OF Anti Il-8 Monoclonal Antibody Secreting CHO DP-12 Cells SOP Volume of CHO DP12 suspension added _____	Operator/Date	Verifier/Date								
10.3. Inoculate bioreactor with CHO DP12 cell suspension per steps 8.4.1 of the Applikon EZ-Control Bioreactor Controller SOP.	Operator/Date	Verifier/Date								
Comments	Operator/Date	Verifier/Date								

Batch Record: Anti IL-8 mAb Production from CHO DP-12 Cells
Lot Number _____

<p>11. Monitoring of Bioreactor Cell Culture. Immediately after inoculation of the bioreactor (Day 0) and at 1 –day intervals, sample the culture to determine OD at 650 nm, viable cell count and viability. 1 mL sample is stored at 4°C for determination of glucose, lactate, and anti IL-8 concentration later.</p>		
<p>11.1 Sampling Procedure Day0-Day2 11.1.1. Label 2 spectrophotometer cuvettes as “blank” and “sample”. 11.1.2. Label 5 microfuge tubes as follows: “cells”, “cell count”, “trypan blue”, “balance”, “anti IL-8 vessel name, day of culture, group initials date”. 11.1.3. Aseptically transfer 1 mL of blank solution from the tube labelled Blank to a microfuge tube labelled “Blank”. 11.1.4. Label 50 mL conical tube “anti- IL8, initial, date”. 11.1.5. Log in to Applikon EZ Controller as operator per Applikon Operator SOP. 11.1.6. Raise the stirrer upper limit to 150 rpm. 11.1.7. Change the stirrer setting to 125 rpm. 11.1.8. Spray the head plate near the sampling tube with 70% IPA. 11.1.9. Remove the black clamp and set on the head plate. 11.1.10. Pull out the autoclavable female connector and set it next to the black clamp. 11.1.11. Place a 10 mL pipette into the sampling tube, remove 25 mL of sample and place sample in a 50 mL conical tube labelled sample. 11.1.12. Put the female autoclavable connector back into the sampling tube. 11.1.13. Bend the sampling tubing and place the black clamp back on the tubing. 11.1.14. Change the stirrer setting to 75 rpm. 11.1.15. Change the stirrer upper limit back to 76 rpm.</p>	Operator/Date	Verifier/Date
<p>Comments</p>	Operator/Date	Verifier/Date

Batch Record: Anti IL-8 mAb Production from CHO DP-12 Cells
Lot Number _____

11.2. Testing Culture Samples- Day 0-Day2		
11.2.1. Record the pH in the data table for bioreactor on page 22-23 of the Batch Record from the EZ-Control Bioreactor Controller display	Operator/Date	Verifier/Date
11.2.2 OD 650nm Measurement 11.2.2.1 Remove 1 mL of sample from the 50 mL conical tube and place in spectrophotometer cuvette labeled “sample”. 11.2.2.2. Remove 1 mL of blank from the microfuge tube and place into spectrophotometer cuvette labelled “Blank”. 11.2.2.3. Measure OD at 650 nm and record the OD in the table on the page 22-23 of the Batch Record. Return 1 mL sample back to the 50 ml conical tube labelled “sample”.	Operator/Date	Verifier/Date
11.2.3. Cell Concentration and Viability –Trypan Blue Assay 11.2.3.1. Centrifuge the 50 mL tube containing sample at 900 rpm for 5 minutes. 11.2.3.2. Carefully remove supernatant, leaving approximately 0.2 mL behind so as not to disturb the pellet. Transfer supernatant to a new 50 mL conical tube. 11.2.3.3. Re-suspend the pellet in 0.8 mL of excess supernatant from step 8.6.2.2.2 using a 5 mL pipette. 11.2.3.4. Perform the trypan blue assay per SOP on the re-suspended pellet. Record the viable cell/ml after correcting for the dilution factor and % viability in the table on the page 22-23 of the Batch Record.	Operator/Date	Verifier/Date
11.2.3. Anti IL-8, Glucose, and Lactate Concentration 11.2.3.1. Remove 1mL of supernatant from 50 mL conical tube in step 8.6.2.2.2 and transfer to a microfuge tube labeled “anti IL-8- bioreactor [time point], [initials], [date]”. 11.3. 3..2. Store sample at 2-8°C in microfuge storage box labeled with date, group name, for measurement of glucose, lactate, and anti-IL8 concentration.	Operator/Date	Verifier/Date

Batch Record: Anti IL-8 mAb Production from CHO DP-12 Cells
Lot Number _____

11.3. Sampling Procedure- Day 3- End of Run (EOR)		
<p>11.3.1. Label 15 mL conical tube “Anti-IL8, initial, date”.</p> <p>11.3.1. Label 2 spectrophotometer cuvettes as “blank” and “sample”.</p> <p>11.3.2. Label 5 microfuge tubes as follows: “cells”, “cell count”, “trypan blue”, “balance”, “anti IL-8-vessel name, day of culture, group initials date”.</p> <p>11.3.3. In BSC, aseptically transfer 1 mL of blank solution from the tube labelled Blank to a microfuge tube labelled “Blank”.</p> <p>11.3.4. Log in to Applikon Bioreactor Controller operator.</p> <p>11.3.5. Raise the stirrer upper limit to 150 rpm.</p> <p>11.3.6. Change the stirrer setting to 125 rpm.</p> <p>11.3.7. Spray the head plate near the sampling tube with 70% IPA.</p> <p>11.3.8. Remove the black clamp and set on the head plate.</p> <p>11.3.9. Pull out the autoclavable female connector and set it next to the black clamp.</p> <p>11.3.10. Place a 10 mL pipette into the sampling tube, remove 5 mL of sample and place sample in a 15 mL conical tube labelled sample.</p> <p>11.1.11. Put the female autoclavable connector back into the sampling tube.</p> <p>11.1.12. Bend the sampling tubing and place the black clamp back on the tubing.</p> <p>11.1.13. Change the stirrer setting to 75 rpm.</p> <p>11.1.14. Change the stirrer upper limit back to 76 rpm.</p> <p>11.3.15. Put the female autoclavable connector back into the sampling tube.</p>	Operator/Date	Verifier/Date
11.4. Testing Culture Samples Day 3- End of Run (EOR)		
<p>11.4.1 Cell Viability</p> <p>11.4.1. Remove 100 µL from 15 mL conical tube containing 3 mL sample and place in microfuge tube labeled “cells count”.</p> <p>11.4.2. Determine viable cell count per Trypan Blue Assay SOP. Record cell viability and concentration in the table on page 22-23 of the Batch Record.</p>	Operator/Date	Verifier/Date

Batch Record: Anti IL-8 mAb Production from CHO DP-12 Cells
Lot Number _____

<p>11.4.2. OD 650 nm Measurement 11.4.2.1. Remove 1 mL of sample from the 15 mL conical tube containing 5 mL sample and place in spectrophotometer cuvette labeled “sample”. 11.4.2.2. Remove 1 mL of blank from the microfuge tube and place in spectrophotometer cuvette labelled “Blank”. 11.4.2.3. Measure OD at 650 nm. Record the OD in the table on page 22-23 of the batch record.</p>	Operator/Date	Verifier/Date
<p>11.4.3 Measurement of Glucose, Lactate, and Anti- IL-8 concentration 11.4.3.1 Remove 1 mL of the remaining 1.9 mL sample and place in the microfuge tube labelled “cells”. Place 1 mL of milliQ water in microfuge tube labeled “balance”. Centrifuge both “cells” and “balance tubes” for 5 minutes in microcentrifuge. 11.4.3.2 Remove supernatant from the sample tube and transfer to microfuge tube labeled “anti- IL-8 vessel name (Spinner or Bioreactor), day of culture, group initials, date”. 11.4.3.3. Store sample at 2-8°C in microfuge storage box labeled with date, group name, for measurement of glucose, lactate, and anti-IL8 concentration. 11.4.3.4 Add 10% bleach solution to the remaining sample and discard in the biohazard waste.</p>	Operator/Date	Verifier/Date
12. Ending a Run		
<p>12.1. Turn off each control loop refer to the Applikon EZ-Control Bioreactor Controller Operation SOP 12.2. Turn off the supply of Air pump. 12.3. Turn off the supply of CO2 tank.</p>	Operator/Date	Verifier/Date
13. Harvest		
<p>13.1. Refer to the SOP: Applikon ez-Control Bioreactor Controller Operation for instructions on removing the head plate of the bioreactor, providing access to the cells and conditioned medium. 13.2. Transfer the culture to three 250 mL centrifuge bottles using a 50 mL pipet and PipetAid. Residual culture can be transferred to an Erlenmeyer flask for temporary storage. 13.3. Centrifuge cells in pre-chilled Sorvall centrifuge, fitted with a SLA1500 rotor, at 500 x g for 5 min, 4°C. 13.4. Transfer conditioned medium (CM) from centrifuge bottle to storage bottle by carefully decanting the supernatant to appropriately labeled 250 mL Corning bottles.</p>	Operator/Date	Verifier/Date

Batch Record: Anti IL-8 mAb Production from CHO DP-12 Cells
Lot Number _____

14. Clean pH and DO Probes		
14.1. Clean the pH and DO probes with DI water. Spray with 70% IPA and pat dry with a lint-free laboratory wipe. 14.2. Store the pH probe in a pH storage solution in a storage bottle. 14.3. Store the DO probe in an electrolyte solution for short term. For long-term storage, store the DO probe dry. Replace the protective cap on the probe.	Operator/Date	Verifier/Date
15. Clean Bioreactor Clean the bioreactor per Applikon EZ-Control Bioreactor Controller Operation SOP.	Operator/Date	Verifier/Date
16. QC Biochemistry of the Samples from spinner and the Bioreactor.		
16.1. Perform the Quantitative Glucose Assay of all the spinner and Bioreactor samples per Glucose Determination Assay SOP.	Operator/Date	Verifier/Date
16.2. Perform the Quantitative Lactate Assay of all the spinner and Bioreactor samples per Lactate Determination Assay SOP.	Operator/Date	Verifier/Date
16.3 Perform the Anti-IL8 Mab Quantitative ELISA Assay of all the spinner and Bioreactor samples per Quantitation of CHO DP-12 Derived Anti IL-8 Monoclonal Antibody by ELISA SOP.	Operator/Date	Verifier/Date
16.4. Prepare the growth curve for spinner flask samples and Bioreactor samples. Spinner Flask Cells/mL, glucose, and lactate vs. time (use 2 y-axes). Anti IL-8 concentration and cells/mL vs. time (use 2 y-axes). Attach graphs to Batch Record. Bioreactor Cells/mL, % viability, and total cells vs. time (use 2 y-axes). Cells/mL, glucose, and lactate vs. time (use 2 y-axes). Anti IL-8 concentration and cells/mL vs. time (use 2 y-axes). Attach graphs to Batch Record.	Operator/Date	Verifier/Date
16.5 Attach QC data to the batch record.	Operator/Date	Verifier/Date
Comments	Operator/Date	Verifier/Date

Batch Record: Anti IL-8 mAb Production from CHO DP-12 Cells

Lot Number _____

Applikon Bioreactor ID # _____

TIME (hours)	OD 650nm	pH	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

Batch Record: Anti IL-8 mAb Production from CHO DP-12 Cells

Lot Number _____

Applikon Bioreactor ID# _____

TIME (hours)	OD 650nm	pH	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

SOP: Labconco Purifier Class 2 Biological Safety Cabinet Operation

Approvals:

Preparer: Jason McMillan

Date: 17JAN14

Reviewer: Dr. Margaret Bryans

Date: 18JAN14

1. Purpose:

1.1. Operation of the Biological Safety Cabinet (BSC).

2. Scope:

2.1. Applies to the use of the BSC for maintaining a sterile environment for media preparation, culture inoculation and culture sampling.

3. Responsibilities:

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

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

4. References:

4.1. Labconco Purifier Class 2 Biological Safety Cabinet Operator's Manual

5. Definitions: N/A

6. Precautions:

6.1. UV Light is damaging to eyes and skin. Avoid exposure.

7. Materials:

7.1. 70% Isopropanol (IPA) in spray bottle

7.2. lab towels

7.3. lab coat

7.4. gloves

8. Procedure:

8.1. UV Decontamination – Performed for initial use of the day

8.1.1. Put on gloves and lab coat.

8.1.2. Open sash **slightly** and **immediately** turn on blower.

8.1.3. Spray and wipe down the stainless steel work surfaces of the BSC with 70% IPA.

8.1.4. With gloved hands, spray all necessary materials not affected by UV with 70% IPA and place in BSC.

8.1.5. Close the sash, turn off the blower, and turn on the UV light. This switch is located on the control panel. Refer to Figure 1.

8.1.6. Leave the UV light on for at least 15 minutes.

8.1.7. Place a biohazard waste receptacle adjacent to the cabinet.

8.1.8. Once the appropriate time has elapsed, turn off the UV light.

8.1.9. Turn on the visible light. This is located on the control panel.

8.1.10. Turn on the receptacle power.

8.1.11. Open the sash **slightly**, and **immediately** turn on the blower.

8.1.12. After blower is on, raise the sash to the safe operating level indicated by a red dot on the left side of the cabinet.

SOP: Labconco Purifier Class 2 Biological Safety Cabinet Operation

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

8.2. Operation

8.2.1. Replace gloves with new ones.

8.2.2. Spray down hands with 70% IPA prior to entering the BSC.

Note: Allow 30 seconds for the 70% IPA to dry. This ensures disinfection.

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

8.2.4. Place all necessary equipment inside of the BSC.

8.2.5. Perform protocol while working in center of the work surface.

Note: **Do not block the intake grills.** This ensures proper airflow. Refer to Figure 3.

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

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

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

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

8.2.10. Turn off the BSC.

9. Attachments:

SOP: Labconco Purifier Class 2 Biological Safety Cabinet Operation

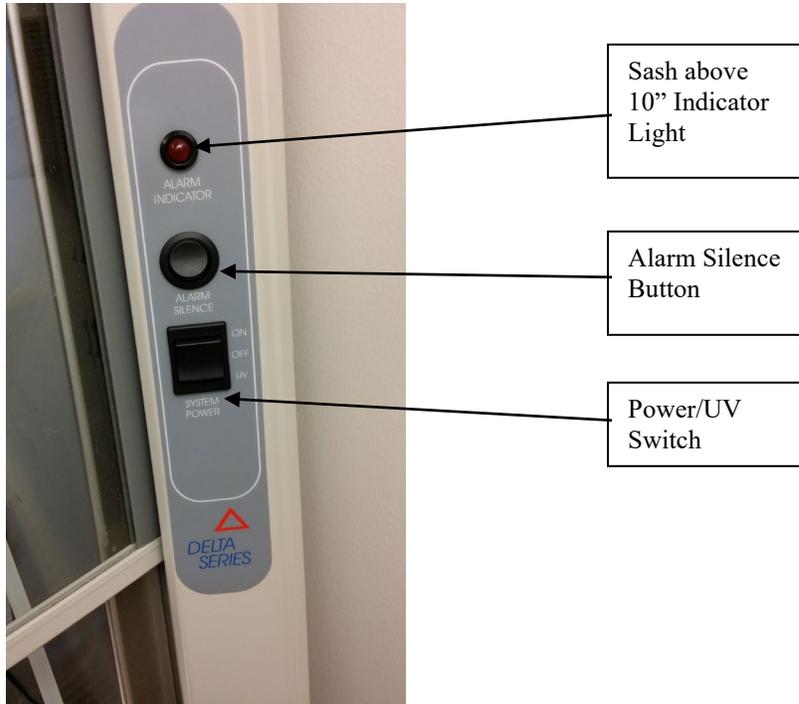
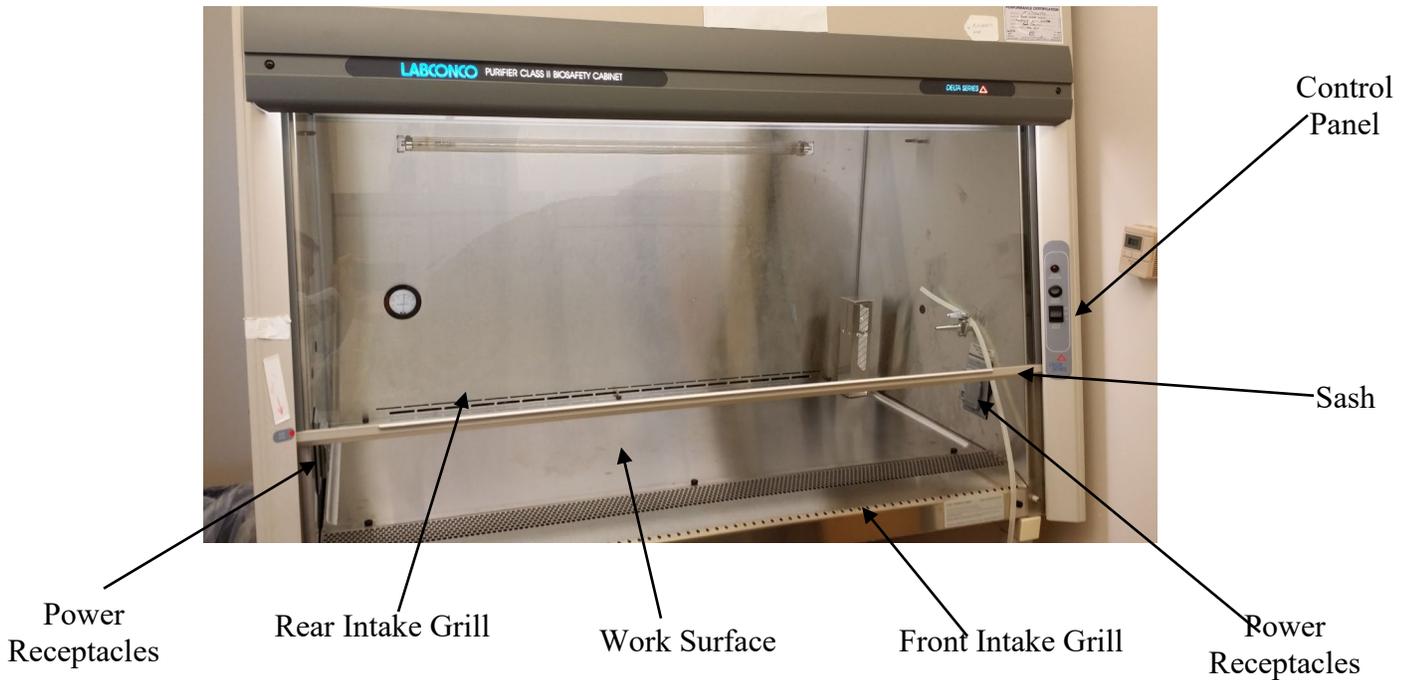


Figure 1: Biological Safety Cabinet Control Panel



SOP: Labconco Purifier Class 2 Biological Safety Cabinet Operation

Figure 2: Biological Safety Cabinet Components



Figure 3: Air Flow Diagram

<http://www.labconco.com/product/purifier-logic-class-ii-type-a2-biosafety-cabinets-2/4261>

10. History:

<i>Revision Number</i>	<i>Effective Date</i>	<i>Preparer</i>	<i>Description of Change</i>
0	17JAN14	Jason McMillan	Initial release

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

Approvals:

Preparer: Dr. Maggie Bryans
Reviewer: Jason McMillan

Date: 03APR09
Date: 03APR09

1. Purpose:

1.1. Cleaning and assembling of the Bellco Spinner Flask (100mL).

2. Scope:

2.1. Applies to the Bellco Spinner Flask (100mL) for maintaining suspension of cultures.

3. Responsibilities:

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

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

4. References:

4.1.1. Bellco Adjustable Hanging Bar Spinner Flask (100mL) manufacturer insert

4.1.2. Autoclave SOP

4.1.3. Bellco Micro-Carrier Spinner Flasks (500mL) SOP

4.1.4. <http://www.bellcoglass.com/>

5. Definitions: N/A

6. Precautions: N/A

7. Materials:

7.1. magnetic stirrer plate

7.2. glassware detergent

7.3. bottle brush

7.4. autoclave

8. Procedure:

8.1. Preparation

8.1.1. Gather all parts: glass spinner flask body, sidearm caps, top cap, compression fitting body, compression fitting retaining nut, cap liner, shaft lock (cap nut, washer and o-ring), glass impeller shaft, magnet and magnet holder.

8.1.2. Wash all parts with a bottle brush, warm water and glassware detergent. Rinse several times with tap water, and then several times with deionized water.

8.2. Assembly

8.2.1. Insert the glass impeller shaft into the hole at the bottom of the magnet holder. The knob at the bottom of the shaft will catch and the magnet holder should spin freely.

8.2.2. Insert magnet into the remaining holes on the magnet holder so that it is centered across the bottom.

8.2.3. Insert cap liner into the top cap. Push the compression fitting body up through the hole in the bottom of the top cap and lock in place using the retainer nut on the top side of the top cap.

8.2.4. Gently slide the glass impeller shaft through the cap from the bottom. Slide the o-ring onto the shaft so that it is flush with the cap liner.

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

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

9. Attachments:

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

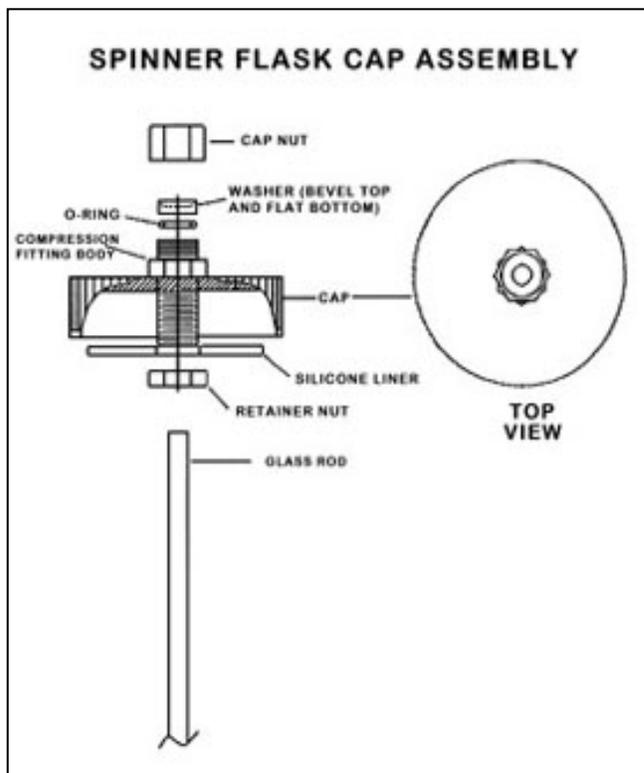


Figure 1: Spinner Flask Cap Assembly
(<http://www.bellcoglass.com/>)

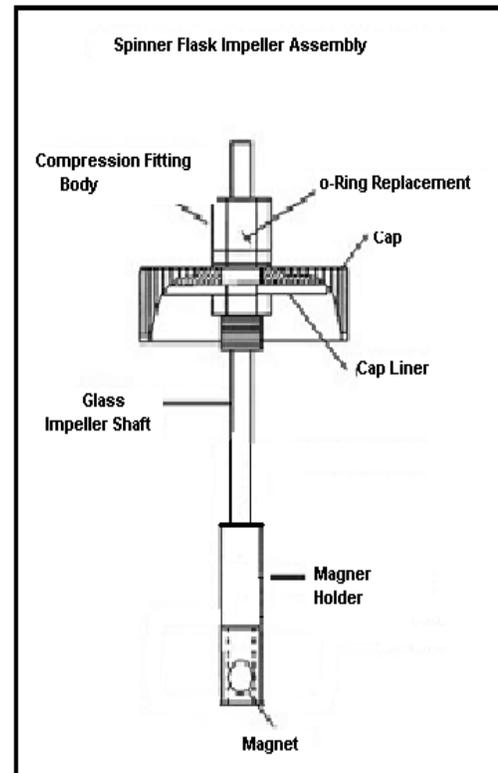


Figure 2: Spinner Flask Impeller Assembly
(<http://www.bellcoglass.com/>)

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

10. History:

Name	Date	Amendment
Jason McMillan	18MAR14	Minor changes

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

Approvals:

Preparer: Jason McMillan

Date 08JAN14

Reviewer: Dr. Margaret Bryans

Date 10JAN14

1. Purpose:

1.1. To calibrate and operate the Oakton PC 700 Bench Series pH/ Conductivity/°C/°F Meter.

2. Scope:

2.1. To measure the pH and conductivity of solutions and media.

3. Responsibilities:

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

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

4. References:

4.1. Oakton Bench 700 Series Quick Guide

4.2. Oakton PC 700 Instruction Manual

5. Definitions: N/A

6. Precautions:

6.1. Use caution when handling all samples due to unknown pH.

6.2. Do not wipe or rub pH electrode. This will create a static build up that will interfere with measurements.

6.3. Always wear the appropriate personal protective equipment (PPE).

7. Materials:

7.1. Oakton PC 700 pH Meter and electrodes

7.2. pH electrode storage solution

7.3. commercially made pH standard buffers as required

7.4. wash bottle

7.5. MilliQ water

7.6. waste beaker

7.7. laboratory tissues, such as Kimwipes

7.8. Oakton 1413 $\mu\text{S}/\text{cm}$ Conductivity Standard

8. Procedure:

8.1. pH Preparation

8.1.1. Select the pH 7 standard buffer and a second standard (and third if needed) buffer that brackets the expected sample pH.

8.1.2. Prepare buffers according to manufacturer's instructions if needed, and ensure that they are not expired.

8.2. pH Calibration

8.2.1. Press the power key to turn on the pH meter.

8.2.2. Gently remove the protective cap/sleeve from the bottom of the pH electrode.

8.2.3. Rinse the pH electrode with MilliQ water and gently blot dry with a laboratory tissue.

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

- 8.2.4. Press the “MODE” key until “pH” is displayed on the right side of primary (top) reading.
- 8.2.5. Dip the pH electrode into the first pH buffer and press the “CAL/MEAS” key until “CAL” is displayed on the top of the LCD display. The secondary (bottom) reading will lock onto the appropriate buffer value. Provide stirring for best results. When the **Ready** indicator appears in the top left portion of the LCD screen, press the “ENTER” key to accept. The primary reading will flash briefly before the secondary reading scrolling the remaining buffer available.
- 8.2.6. Rinse the pH electrode with MilliQ water, gently blot dry with a Kimwipe, and then dip the pH electrode into the second pH buffer. The secondary reading will lock onto the appropriate buffer value. When the **READY** indicator appears, press the “ENTER” key to accept. The primary reading will flash briefly and then display the percent efficiency (slope) before the secondary reading begins scrolling the remaining available buffers.
- 8.2.7. Press the “CAL/MEAS” key to return to measurement mode or to calibrate a third buffer repeat step 8.2.6.
- 8.2.8. Remove the pH electrode from the solution, rinse the pH electrode with MilliQ water and blot dry with a laboratory tissue.
- 8.2.9. Replace storage cap if immediate measurement of sample is not needed.

8.3. pH Measurement

- 8.3.1. If necessary, gently remove the protective cap/sleeve from the bottom of the pH electrode, rinse the pH electrode with MilliQ water and blot dry with laboratory tissue. Be sure **MEAS** is visible on the top left side of the LCD display
- 8.3.2. Insert pH electrode into the sample. Provide stirring.
- 8.3.3. Read pH value on the primary reading when the value has stabilized.
- 8.3.4. Remove the pH electrode from the solution, rinse the pH electrode with MilliQ water and blot dry with a laboratory tissue.
- 8.3.5. Repeat steps 8.3.2. through 8.3.4. for additional samples.
- 8.3.6. When finished, replace protective cap onto end of the pH electrode and turn off the pH meter by pressing the power key.

8.4. Automatic Conductivity Calibration

- 8.4.1. Press the “MODE” key as needed to select conductivity (μS or mS).
- 8.4.2. Dip the conductivity electrode into the selected conductivity standard beyond the upper steel band (utilize the fill line on the outside of the probe guard for reference) and press the “CAL/MEAS” key until “CAL” is visible on the LCD display. Provide stirring.
- 8.4.3. The primary reading will show the factory default value, while the secondary reading will lock on the appropriate automatic standard value from Table 1.
- 8.4.4. When the **READY** indicator appears, press the “ENTER/RANGE” key to accept. The primary reading will flash briefly before returning to measurement mode upon successful calibration.

8.5. Conductivity Measurement

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

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

laboratory tissue.

- 8.5.2. Dip the conductivity electrode into the sample beyond the upper steel band (utilize the fill line on the outside of the probe guard for reference).
- 8.5.3. Allow time for the reading to stabilize on the primary reading. **The clear yellow protective probe guard must be attached during measurement to prevent erroneous results.**
- 8.5.4. Rinse the conductivity electrode with MilliQ water and gently shake off any excess water droplets.
- 8.5.5. Turn off the pH/Conductivity meter by pressing the power key.

9. Attachments:

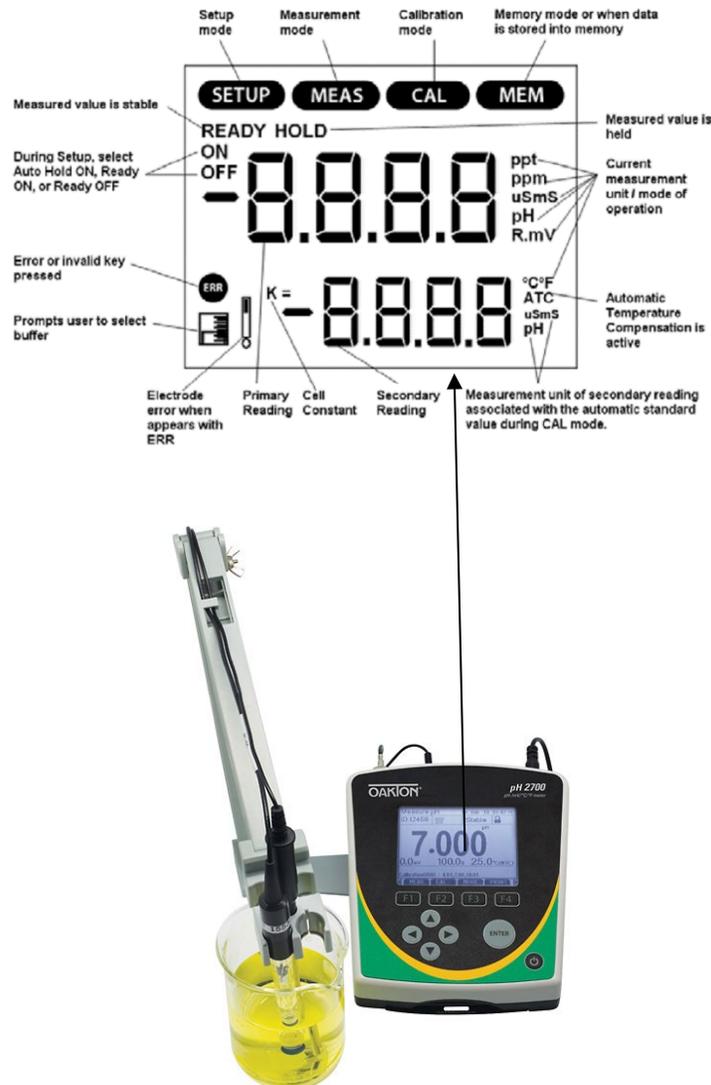
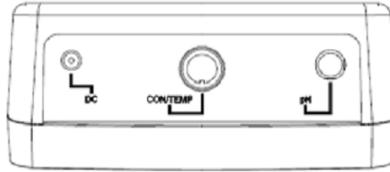


Figure 1: Oakton PC 700 LCD Annunciators
(http://static.coleparmer.com/large_images/35420_20a.jpg)

SOP: Oakton PC 700 Bench Series pH/ Conductivity/ $^{\circ}$ C/ $^{\circ}$ F Meter

Oakton PC 700 Manual



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

Figure 2: Meter Connections
Oakton PC 700 Manual

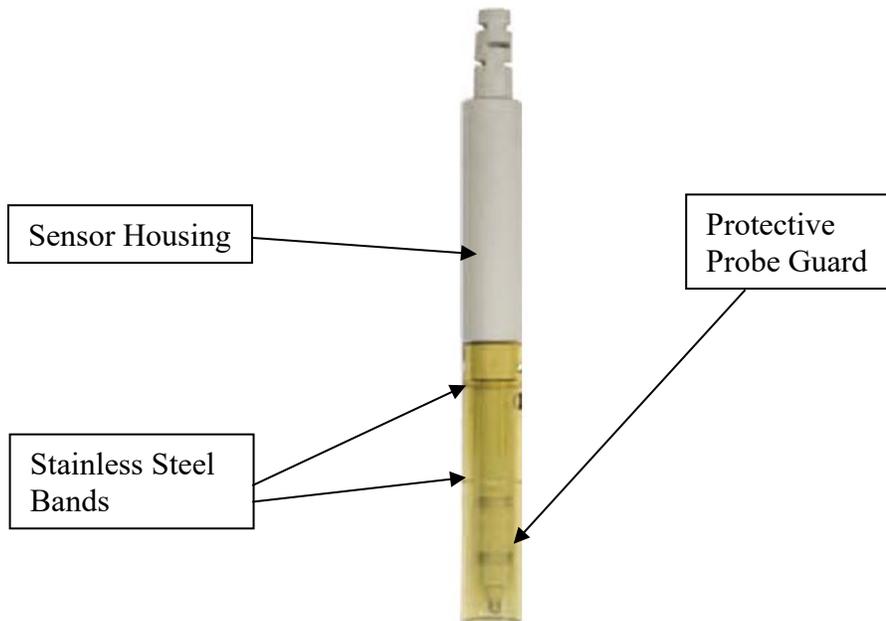


Figure 3: Conductivity Electrode
Oakton PC 700 Manual

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

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

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

Table 1: Conductivity Calibration Standard values
 Oakton PC 700 Manual

10. History:

<i>Revision Number</i>	<i>Effective Date</i>	<i>Preparer</i>	<i>Description of Change</i>
0	08JAN14	Jason McMillan	Initial release

SOP: Applikon EZ-Control Bioreactor Controller Operation

Approvals

Preparer: John Buford	Date: 19DEC13
Reviewer: Jason McMillan	Date: 16SEP14
Reviewer: Hetal Doshi	Date: 8JULY17
Reviewer: Dr. Maggie Bryans	Date: 10JULY17

1. Purpose

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

2. Scope and Applicability

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

3. Summary of Method

- 3.1. Preparing the controller
 - 3.1.1. Power up the controller and login
 - 3.1.2. Enter the project name (optional)
 - 3.1.3. Emergency stop and resume (emergency only)
- 3.2. Preparing the bioreactor
 - 3.2.1. Fill the bioreactor with culture medium or 100ml 1X PBS
 - 3.2.2. Calibrate the pH sensor
 - 3.2.3. Mount sensors to the bioreactor
 - 3.2.4. Prepare the liquid addition bottles
 - 3.2.5. Mount connections to the bioreactor
 - 3.2.6. Autoclave the bioreactor and addition bottles
- 3.3. Connecting the bioreactor to the controller
 - 3.3.1. Verify that all control loops are switched off
 - 3.3.2. Connect the sensors
 - 3.3.3. Connect the heating blanket
 - 3.3.4. Connect the aeration
 - 3.3.5. Connect the stirrer motor
 - 3.3.6. Connect the alkaline bottle
 - 3.3.7. Enter process parameter settings
 - 3.3.8. Start all control loops
 - 3.3.9. Calibrate the DO sensor
 - 3.3.10. Reset dose monitor values
- 3.4. Starting the cultivation

SOP: Applikon EZ-Control Bioreactor Controller Operation

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

4. References

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

5. Precautions

- 5.1. Alkaline solutions used for controlling pH are caustic. Read the Material Safety Data Sheet (MSDS) for hazards, handling and storage information. Wear personal protection equipment (PPE).
- 5.2. Do not put the stirrer motor or the heating blanket in the autoclave.
- 5.3. The O₂ electrolyte solution used in 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. Responsibilities

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

SOP: Applikon EZ-Control Bioreactor Controller Operation

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

7. Equipment and Materials

- 7.1. Applikon 3-liter glass autoclavable bioreactor:
 - 7.1.1. Vessel
 - 7.1.2. Head plate
 - 7.1.3. Gas sparger (pre-mounted on the head plate)
 - 7.1.4. Impeller (pre-mounted on the head plate)
 - 7.1.5. Air outlet condenser (pre-mounted on the head plate)
 - 7.1.6. Septum (pre-mounted on the head plate)
 - 7.1.7. Sample pipe (pre-mounted on the head plate)
 - 7.1.8. pH sensor
 - 7.1.9. DO sensor
 - 7.1.10. Temperature sensor
 - 7.1.11. Stirrer motor
 - 7.1.12. Heating blanket
- 7.2. Applikon ez-Control bioreactor controller
- 7.3. Laboratory gasses:
 - 7.3.1. Air compressor
 - 7.3.2. O₂ (optional)
 - 7.3.3. CO₂
- 7.4. pH 4.0 and pH 7.0 buffer standards
- 7.5. Liquid addition bottles
- 7.6. Alkaline solution (generally 1M sodium bicarbonate)
- 7.7. Gas filters, 0.2 µm
- 7.8. Autoclavable silicone tubing, size 14 (1.6 mm interior diameter)
- 7.9. Autoclavable silicone tubing, size 16 (3.1 mm interior diameter)
- 7.10. Autoclavable silicone tubing, size 25 (4.8 mm interior diameter)
- 7.11. Tubing clamps
- 7.12. Cotton and aluminum foil (for autoclaving)
- 7.13. Autoclave indicator tape
- 7.14. Culture medium
- 7.15. Culture inoculum
- 7.16. Autoclave (such as a Tuttnauer 3850 ELV)
- 7.17. Computer system with BioXpert Lite installed
- 7.18. Autoclavable male and female connectors

8. Procedure

- 8.1. Preparing the controller
 - 8.1.1. Power up the controller and login

SOP: Applikon EZ-Control Bioreactor Controller Operation

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

- 8.1.1.1. Power up the controller using the green power switch located on the back of the controller (upper right).
- 8.1.1.2. The touch screen display located on the front shows that the controller is initializing. Once initialization completes, the display switches to the Home screen.
- 8.1.1.3. Login as Operator using the touch screen display:
 - 8.1.1.3.1. Home > login button (top middle) > Operator > Login
 - 8.1.1.3.2. Enter the Operator password: 0000
- 8.1.1.4. To logout: Home > login button (top middle) > View *Note: logout is automatic after a period of inactivity (generally 10 minutes).*
- 8.1.1.5. The touch screen backlight is switched off automatically after a period of inactivity (generally 30 minutes) and the touch screen goes dark. To switch on the backlight, touch the screen.

8.1.2. Enter the project name (optional)

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

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

8.1.3. Emergency stop and resume (emergency only)

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

- 8.1.3.1. In the event of an emergency requiring the bioreactor process to stop immediately, press the red Emergency Stop button located on the front of the controller.
- 8.1.3.2. To resume the bioreactor process:
 - 8.1.3.2.1. Remove the cause of the Emergency Stop.
 - 8.1.3.2.2. Turn the Emergency Stop button clockwise until it resets itself to the normal position.
 - 8.1.3.2.3. Login as System Engineer.

SOP: Applikon EZ-Control Bioreactor Controller Operation

8.1.3.2.4. Verify that the bioreactor is in a safe condition.

8.1.3.2.5. Select Resume on the Emergency Stop screen to restart the control loops.

8.2. Preparing the bioreactor

8.2.1. Fill the bioreactor with culture medium

The bioreactor is filled with culture medium before it is autoclaved (if it will not damage the media) so that the medium is sterilized along with the bioreactor. Do not exceed the working volume of the bioreactor (2.4 liters for a 3-liter bioreactor). Leave enough space for inoculation and nutrients to be added during cultivation. For mammalian cell culture the media is added to the bioreactor after autoclaving. 100 ml of 1XPBS is added to the bioreactor before autoclaving.

8.2.1.1. A process SOP should provide details regarding culture medium composition and volume. E.g. refer to section 8.5 of Batch Culture of anti-IL-8 Monoclonal Antibody secreting CHO DP-12 Cells

8.2.1.2. Loosen the six mill nuts that fastens the head plate on the bioreactor vessel and remove the head plate.

8.2.1.3. Add culture medium or 100ml of 1X PBS. Refer to the process SOP for the details.

8.2.1.4. Refer to the process Batch Record for assembling of the bioreactor.

8.2.1.5. Mount the head plate on top of the vessel and fasten with the six mill nuts finger-tight.

8.2.2. Calibrate the pH sensor

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

8.2.2.1. Measure (and record in Batch Record) the temperature of the pH buffer standards using a thermometer.

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

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

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

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

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

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

8.2.2.8. Touch the button 2-point calibration.

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

SOP: Applikon EZ-Control Bioreactor Controller Operation

- 8.2.2.10. When prompted for the pH value of the first buffer solution, put the pH sensor in the pH 4.0 buffer standard and wait until the shown process value stabilizes (shown near the Cancel button). Enter the pH value using the numeric keypad.
 - 8.2.2.11. Rinse the pH sensor and repeat using the pH 7.0 buffer standard. Again, wait for the shown process value to stabilize and enter the corresponding pH.
 - 8.2.2.12. Return to the pH Calibration screen to verify the newly found calibration data (slope and offset). Record slope and offset in the Batch Record.
- 8.2.3. Mount sensors to the bioreactor
pH and DO sensors are mounted to the bioreactor before it is autoclaved so that the sensors are sterilized along with the bioreactor. Some systems also use a foam / level sensor.
- 8.2.3.1. Disconnect the cable of the pH sensor.
 - 8.2.3.2. Cover the pH sensor connector with the pH sensor screw cap. Verify that the rubber gasket is in place between the sensor connector and the cap.
 - 8.2.3.3. Insert the pH sensor into its port in the head plate and fasten it. See Figure 6 for the location of the pH sensor port.
 - 8.2.3.4. Remove the protective cap from the bottom of the DO sensor.
 - 8.2.3.5. Inspect the screen at the bottom of the probe tip. Replace if damaged.
 - 8.2.3.6. Holding the probe in a vertical position, unscrew module from the bottom of the probe
 - 8.2.3.7. Inspect the integrity of the O-ring underneath the module and replace if worn or cracked
 - 8.2.3.8. Replenish DO electrolyte solution. There should be 1 ml of electrolyte solution in the membrane module.
 - 8.2.3.9. Screw the membrane module to the probe
 - 8.2.3.10 Repeat steps 8.2.3.2 and 8.2.3.3 for the DO sensor.
- 8.2.4. Prepare the liquid addition bottles
Liquid addition bottles are used to add liquids to the bioreactor aseptically (see Figure 7). For example, Media is added, alkaline solution is added to raise pH and inoculum is added to begin cultivation. Addition bottles are sterilized along with the bioreactor.
- 8.2.4.1. A process SOP should provide the information regarding the alkaline solution composition and water.
 - 8.2.4.2. Fill a liquid 250ml addition bottle with alkaline solution, no more than 2/3 (150ml) full so that it can be autoclaved. Cap the alkaline bottle with a two-port top.
 - 8.2.4.3. Connect the air inlet on the alkaline bottle to a gas filter using a short length (approx. 7 cm) of size 25 tubing. Do not clamp.
 - 8.2.4.4. Add 5 mL laboratory grade water to a 500ml addition bottle to be used for transferring inoculum. This will improve the heat transfer during

SOP: Applikon EZ-Control Bioreactor Controller Operation

sterilization in the autoclave. Cap the inoculum transfer bottle with a two-port top.

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

8.2.4.6. Connect the liquid outlet on the inoculum transfer bottle to an autoclavable male connector using a long length (approx. 75 cm) of size 25 tubing.

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

8.2.4.8 Repeat steps 8.2.4.4. to 8.2.4.7 with 1L media addition bottle.

8.2.5. Mount connections to the bioreactor

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

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

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

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

8.2.5.4. Connect the sparger inlet to a gas filter using approx. 60 cm length of size 25 tubing. Do not clamp.

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

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

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

8.2.5.8. Cover all tubing and head plate with aluminum foil.

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

8.2.6. Autoclave the bioreactor and liquid addition bottles

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

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

SOP: Applikon EZ-Control Bioreactor Controller Operation

- 8.2.6.2. Place the assembled bioreactor, the alkaline bottle without disconnecting tubing, 1L addition bottle with tubing and 500ml Inoculum transfer bottle with tubing in the autoclave.
 - 8.2.6.3. Loosen the caps on the alkaline bottle, liquid addition bottle and the inoculum transfer bottle.
 - 8.2.6.4. Close the autoclave and run it on the liquid cycle with slow exhaust option per the autoclave SOP.
 - 8.2.6.5. When the cycle completes, allow the autoclave to cool gradually. Do not open the autoclave until the temperature in the autoclave has dropped below 90°C. After reaching that temperature, open the autoclave to allow it to cool down until the contents can be unloaded safely.
 - 8.2.6.6. Tighten the caps on the alkaline bottle and the inoculum transfer bottle.
 - 8.2.6.7. Remove the assembled bioreactor and the alkaline bottle together and place beside them on the right side of the controller without disconnecting the tubing.
 - 8.2.6.8. Remove the inoculum transfer bottle and place it in a biological safety cabinet.
 - 8.2.6.9. Allow to cool to room temperature.
 - 8.2.6.10. Perform a visual inspection to verify that the autoclave indicator tape changed color and that the bioreactor is dry.
 - 8.2.6.11. Remove aluminum foil from gas filters on the bioreactor and alkaline bottle. Leave the foil on the inoculum transfer bottle and the addition bottle.
 - 8.2.6.12. Place the bioreactor and alkaline bottle on the right side of the controller.
- 8.3. Connecting the bioreactor to the controller
- After the assembled bioreactor is autoclaved, it must be connected to the controller.*
- 8.3.1. Verify that all control loops are switched off
 - 8.3.1.1. Login as Operator per section 8.1.1.3 if not already logged in.
 - 8.3.1.2. Verify that all control loops are switched off. The Process Value buttons (bottom of Home Screen) should be gray or yellow. If necessary, stop controllers: Home > Menu (top right) > Start/Stop all controllers > Stop all controllers
 - 8.3.2 Addition of the media to the Bioreactor

If mammalian cells are used for the cell culture production bioreactor is autoclaved without addition of the media. The media is added to the bioreactor using the 1L addition bottle.

 - 8.3.2.1. Refer to the Batch culture SOP for the details regarding the media composition
 - 8.3.2.2. Spray or swab the required material with 70% ethanol, and place in the biological safety cabinet
 - 8.3.2.3. Aseptically add the media component to the prepared 1L addition bottle with the tubing and autoclavable male connector attached.

SOP: Applikon EZ-Control Bioreactor Controller Operation

- 8.3.2.4. Be sure the cap is on tightly and remove the 1L addition bottle with tubing and autoclavable male connector attached and bring it over to Applikon bioreactor.
 - 8.3.2.5. Carefully remove the foil from the female connector on the sample port of the Applikon bioreactor.
 - 8.3.2.6. Carefully remove the aluminum foil from the male connector on the 1L addition bottle and connect the male connector to the female connector on the sample port of the bioreactor.
 - 8.3.2.7. Remove the clamp on the female connector on the addition port of the Applikon bioreactor.
 - 8.3.2.8. On the Applikon reactor touch screen select Menu. Manual Control > Acid Pump On.
 - 8.3.2.9. As the pump turns feed the tubing around it. Use care to avoid pinching fingers.
 - 8.3.2.10. Once all of the media has been transferred into vessel, turn off the acid pump. On the Applikon screen select Menu > Manual control > Acid Pump off.
 - 8.3.2.11. Disconnect the male connector of the addition bottle from the female connector on the sample port of the bioreactor. Bend the tubing of the addition port and reattach the clamp.
- 8.3.3. Connect the sensors
Sensors are connected electrically to the controller.
- 8.3.3.1. Place the bioreactor and alkaline bottle on the right side of the controller if they are not already.
 - 8.3.3.2. Remove the pH sensor screw cap. Connect the pH sensor to the pH sensor cable on the right side of the controller. Verify that the pH sensor cable is plugged into the controller correctly.
 - 8.3.3.3. Remove the DO sensor screw cap. Connect the DO sensor to the DO sensor cable on the right side of the controller. Verify that the DO sensor cable is plugged into the controller correctly.
 - 8.3.3.4. Insert the temperature sensor into the thermometer pocket. Verify that the temperature sensor cable is plugged into the controller correctly. Fill the thermometer pocket with MilliQ water in order to decrease the dead time of the sensor and make temperature control more accurate.
- 8.3.4. Connect the heating blanket
An electric heating blanket is used for warming the bioreactor as needed. Some systems also use a thermo circulator and cooling water for cooling the bioreactor.
- 8.3.4.1. Wrap the heating blanket around the bioreactor vessel (around the glass and inside the support legs). Position the blanket so that the volume markings on the vessel are visible. Fasten the blanket in place using the Velcro ends of the blanket.
 - 8.3.4.2. Verify that the heating blanket is plugged into the controller correctly.

SOP: Applikon EZ-Control Bioreactor Controller Operation

8.3.5. Laboratory gasses (air, O₂, and CO₂) are added by the controller to the bioreactor sparger in order to control DO and to lower pH as needed.

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

8.3.5.2. Open the CO₂ tank and set its regulator to 10 psi.

8.3.6. Connect the stirrer motor

The stirrer motor and the impeller are used to control agitation of the cell culture.

When connecting the stirrer motor, it is helpful for the stirrer to be on.

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

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

8.3.6.3. Touch the alarm limit button > Lower alarm limit > enter 58. > Touch the stirrer set point, enter 60.

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

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

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

8.3.7. Connect the alkaline bottle

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

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

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

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

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

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

8.3.7.6. Turn the pump off.

8.3.7.7. Clip the tubing into the upper pump clamp.

8.3.7.8. Close the pump cover.

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

8.3.8. Enter process parameter settings

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

SOP: Applikon EZ-Control Bioreactor Controller Operation

- 8.3.8.1. A process SOP should provide details regarding process setpoints and alarm limits.
- 8.3.8.2. Login as Operator per section 8.1.1.3 if not already logged in.
- 8.3.8.3. Go to the pH Settings screen: Home > pH (bottom)
- 8.3.8.4. Enter the process settings for the pH control loop:
 - 8.3.8.4.1. Enter the pH upper alarm limit: pH settings > Alarm limits > Upper alarm limit Enter the pH upper alarm limit value provided by the process SOP.
 - 8.3.8.4.2. Enter the pH lower alarm limit: pH settings > Alarm limits > Lower alarm limit Enter the pH lower alarm limit value provided by the process SOP.
 - 8.3.8.4.3. Enter the pH setpoint: pH settings > pH setpoint Enter the pH setpoint value provided by the process SOP.
 - 8.3.8.4.4. Enter any additional pH PID controls provided by the process SOP: pH settings > pH controller setup
- 8.3.8.5. Repeat for temperature and stirrer control loops.
 - 8.3.8.5.1. For stirrer control loop, change set point from 60 to 73, then change lower limit.
- 8.3.9. Start the pH controller loop, temperature controller loop and Stirrer controller loop. Don't start the DO controller Loop until the DO probe is calibrated.
The DO sensor needs to be polarized for at least 6 hours and the temperature and pH need to be stabilized at set point before the DO sensor can be calibrated.
 - 8.3.9.1. Login as Operator per section 8.1.1.3 if not already logged in.
 - 8.3.9.2. Start pH control loop: Home > pH (bottom)>Start pH control loop
 - 8.3.9.3. Start temp control loop: Home > temp (bottom) >Start temp Loop
 - 8.3.9.4. Start Stirrer control loop: Home> stirrer (bottom)> Start stirrer Loop.
 - 8.3.9.5. Allow the process to run for at least 6 hours.
- 8.3.10. Calibrate the DO sensor
The DO measurement is based on the polarographic principle (Clark-cell). Therefore, the sensor must be polarized for at least 6 hours before it can be calibrated.
 - 8.3.10.1. Start the air pump. Set the pressure at 10 psi.
 - 8.3.10.2. Login as Operator per section 8.1.1.3 if not already logged in.
 - 8.3.10.3. Verify that the medium in the bioreactor is stable at process temperature.
 - 8.3.10.4. Stop the DO control loop: Home > DO (bottom) > Stop DO controller
 - 8.3.10.5. Go to the DO Calibration screen: Home > DO > Calibrate DO The numerical data for Slope and Offset are displayed.
 - 8.3.10.6. Verify if the measuring range is set to Air. If not, set it for air: Calibrate DO > Set measurement range > Measurement range for air
 - 8.3.10.7. Open the aeration valve manually: Home > DO > Manual control > O2 Valve: On

SOP: Applikon EZ-Control Bioreactor Controller Operation

- 8.3.10.8. Continue aeration until DO reading is stable (15 to 20 minutes).
- 8.3.10.9. Close the aeration valve manually: Home > DO > Manual control > O2 Valve: Off
- 8.3.10.10. Set the DO calibration value to 100%: Home > DO > Calibrate DO > Calibrate > Enter Calibration Value > 100
- 8.3.10.11. Return to the DO Calibration screen to verify the newly found calibration data (slope and offset). The expected slope value of the sensor (for measurement range for air) is:
 - 2.0 to 4.0 at 25 °C
 - 1.5 to 3.0 at 37 °C
- 8.3.10.12. Start the DO control loop: DO (bottom) > Start DO controller.

8.3.11. Reset dose monitor values

When all control loops are at set-point, the bioreactor system is ready for cultivation (inoculation). All Dose Monitor values should be reset to 0 ml.

- 8.3.11.1. Go to the Home screen and verify that the control loops are on. Allow the process to run until all control loops are at set-point.
- 8.3.11.2. Reset all dose monitor values: Home > Menu > Dose Monitor > Reset all dose monitors > Are you sure? Yes

8.4. Starting the cultivation

8.4.1. Inoculate the bioreactor

Once the process parameters in the bioreactor are at their setpoints, the inoculum is added to the bioreactor aseptically. This SOP makes use of a sterile addition bottle for this purpose.

- 8.4.1.1. Fill the sterile inoculum transfer bottle with inoculum using aseptic technique per the biosafety cabinet SOP. Do not remove the foil from the gas filter or the autoclavable male connector. Recap the bottle before removing it from the biosafety cabinet. Place the inoculum bottle on the right side of the controller.
- 8.4.1.2. Login as Operator per section 8.1.1.3 if not already logged in.
- 8.4.1.3. Stop all control loops: Home > Menu > Start/Stop all controllers > Stop all controllers
- 8.4.1.4. Swab gloves with 70% isopropanol; spray the female connector on the sample port.
- 8.4.1.5. Remove the foil from the attached tubing connected to the male connector and the gas filter on the inoculum transfer bottle. Connect the female connector on the sample port of the bioreactor to the male connector of the inoculum bottle.
- 8.4.1.6. Remove the clamp on the female connector on the addition port of the Applikon bioreactor.
- 8.4.1.7. On the Applikon bioreactor touch screen select Menu. Manual Control > Acid Pump ON

SOP: Applikon EZ-Control Bioreactor Controller Operation

- 8.4.1.8. As the pump turns feed the tubing around it. Use care to avoid pinching fingers.
- 8.4.1.9. Once all of the cell suspension has been transferred into vessel, turn off the acid pump. On the Applikon screen select Menu > Manual control > Acid pump Off
- 8.4.1.10. Disconnect the male connector of the addition bottle from the female connector on the sample port of the bioreactor. Bend the tubing of the addition port and reattach the clamp.
- 8.4.1.11. Re-start all control loops: Home > Menu > Start/Stop all controllers > Start all controllers.

8.5. Monitoring the cell culture

8.5.1. View process data

Process values for the last 72 hours are stored in controller memory and can be displayed using the Trend View screen. Process values for the entire run are stored and displayed using BioXpert Lite.

8.5.1.1. To view process data for a specific process parameter using the Trend View:

- 8.5.1.1.1. Login as Operator per section 8.1.1.3 if not already logged in.
- 8.5.1.1.2. Home > process value button (2nd row from the bottom)
- 8.5.1.1.3. Touch the X-axis button (bottom left) in order to change the range of the time axis (between 1 and 72 hours).
- 8.5.1.1.4. Touch the Y-axis upper limit button (top right) or lower limit button (bottom right) in order to change the range of the process value axis.

8.5.2. Sample the cell culture

Periodically, the cell culture is sampled in order to measure cell concentration and perform product assays per the process SOP. Some bioreactors include a sample system connected to the sample pipe which enables cell culture to be drawn into a sample bottle that can then be replaced aseptically. This system simply uses a pipette to draw cell culture from the sample pipe.

- 8.5.2.1. Refer to the Batch Record for the amount of sample to be withdrawn. Locate the clamped tubing connected to the sample pipe on the bioreactor head plate. Connect a pipette to the sample pipe tubing.
- 8.5.2.2. Release the clamp, withdraw cell culture, and re-clamp. (Sample pipe volume = 4mm ID X 25 cm = 3.2 cm³ = 3.2 mL). Use this sample for cell counts and product assays per the process SOP.

8.6. Harvesting the cell culture

A process SOP should specify when the cell culture is to be harvested.

8.6.1. Stop the controllers

- 8.6.1.1. Login as Operator per section 8.1.1.3 if not already logged in.
- 8.6.1.2. Stop all control loops: Home > Menu > Start/Stop all controllers > Stop all controllers
- 8.6.1.3. Close the CO₂ tank.

SOP: Applikon EZ-Control Bioreactor Controller Operation

8.6.1.4. Turn off the air compressor.

8.6.2. Disconnect the bioreactor

The bioreactor must be disconnected from the controller so that the bioreactor can be decanted.

8.6.2.1. Locate the tubing that connects the alkaline bottle to the bioreactor.

Clamp the tubing near the bioreactor. Disconnect the tubing from the alkaline bottle and remove the tubing from the controller pump.

8.6.2.2. Lift the stirrer motor from the bioreactor head plate and set the motor aside.

8.6.2.3. Disconnect the gas filter on the bioreactor sparger inlet from the tubing to the aeration outlet of the controller.

8.6.2.4. Unwrap the heating blanket from around the bioreactor vessel and set the blanket aside being sure it is lying flat.

8.6.2.5. Disconnect the pH sensor cable from the pH sensor. Cover the pH sensor connector with the pH sensor screw cap.

8.6.2.6. Repeat step 8.6.2.5 for the DO sensor.

8.7. Cleaning the bioreactor

8.7.1. Clean and store the sensors

8.7.1.1. Remove pH and DO sensors from the bioreactor head plate.

8.7.1.2. Rinse the pH and DO sensors thoroughly with MilliQ water, being careful to remove all broth-residue. Gently pat dry with a clean lint-free laboratory wipe. Spray with 70% IPA and gently pat dry with a clean lint-free laboratory wipe

8.7.1.3.. Rinse with MilliQ water and pat dry with a clean lint-free laboratory wipe.

8.7.1.4. Fill the protective cap of the pH sensor 1/2 full with 3M potassium chloride (KCl) solution. Cover the tip of the pH sensor with its protective cap. Verify that the pH electrode is completely immersed in KCl solution.

8.7.1.5. Cover the tip of the DO sensor with its protective cap. The DO sensor can be stored in an electrolyte solution upright for short term. For long term storage store dry.

8.7.1.6. Cover the pH sensor connector with the pH sensor screw cap. Repeat for the DO sensor.

8.7.2. Decant the cell culture

8.7.2.1. Place the disconnected bioreactor in a biosafety cabinet if specified by the process SOP.

8.7.2.2. Loosen the six mill nuts that fasten the head plate on the bioreactor vessel and remove the head plate.

8.7.2.3. Decant the cell culture into a suitable container per the process SOP.

8.7.2.4. Re-mount the head plate on top of the vessel and fasten with the six mill nuts finger-tight.

8.7.3. Pre-clean the bioreactor in place

SOP: Applikon EZ-Control Bioreactor Controller Operation

- 8.7.3.1. Fill the bioreactor with a working volume of 0.1M NaOH solution (2.4 liters for a 3-liter bioreactor).
- 8.7.3.2. Connect the stirrer motor per section 8.3.6.
- 8.7.3.3. Activate the stirrer at 250 RPM for 30 minutes. Visual check for dissolution of foam, debris and other contamination in the bioreactor.
- 8.7.3.4. Stop the stirrer. Lift the stirrer motor from the bioreactor head plate and set the motor aside.
- 8.7.3.5. Drain the bioreactor.
- 8.7.4. Disassemble the bioreactor and clean all parts
 - 8.7.4.1. Remove all tubing and gas filters from the bioreactor head plate assembly and discard in biohazardous waste.
 - 8.7.4.2. Remove the septum from the head plate.
 - 8.7.4.3. Remove the air outlet condenser from the head plate and disassemble the condenser for cleaning.
 - 8.7.4.4. Remove the head plate from the bioreactor vessel.
 - 8.7.4.5. Clean all parts carefully and thoroughly using a small soft bristle brush (e.g. tooth brush) and a dilute laboratory glassware cleaner. Rinse with MilliQ water and then repeat with a 10% bleach solution. Rinse thoroughly with MilliQ water and spray with 70% IPA and place on paper towels on a lab bench to dry.
 - 8.7.4.6. Let dry all the parts.
- 8.7.5. Clean the porous sparger tip (optional)

Depending on the type of medium that is used (presence of proteins and/or peptides), the porous sparger tip may require a special cleaning procedure.

 - 8.7.5.1. Remove the sparger tip from the air inlet pipe.
 - 8.7.5.2. Soak the sparger overnight in a solution of 10 mg/mL pepsin / 0.01M HCl.
 - 8.7.5.3. Use ultrasonic cleaning with water and/or ethanol.
 - 8.7.5.4. Replace the sparger tip onto the air inlet pipe.

SOP: Applikon EZ-Control Bioreactor Controller Operation

9. Attachments

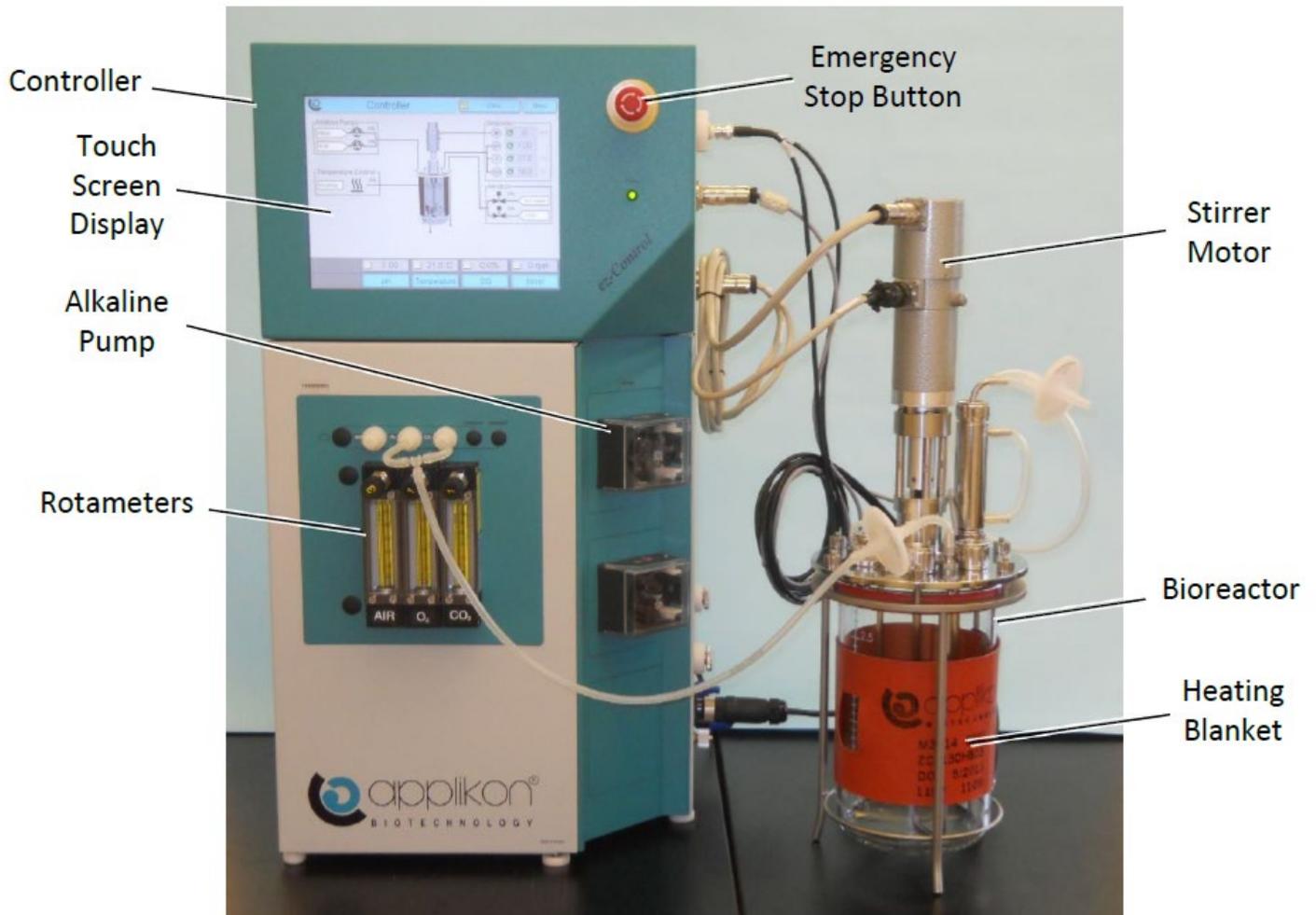


Figure 1. Applikon ez-Control Bioreactor Controller and Bioreactor

SOP: Applikon EZ-Control Bioreactor Controller Operation

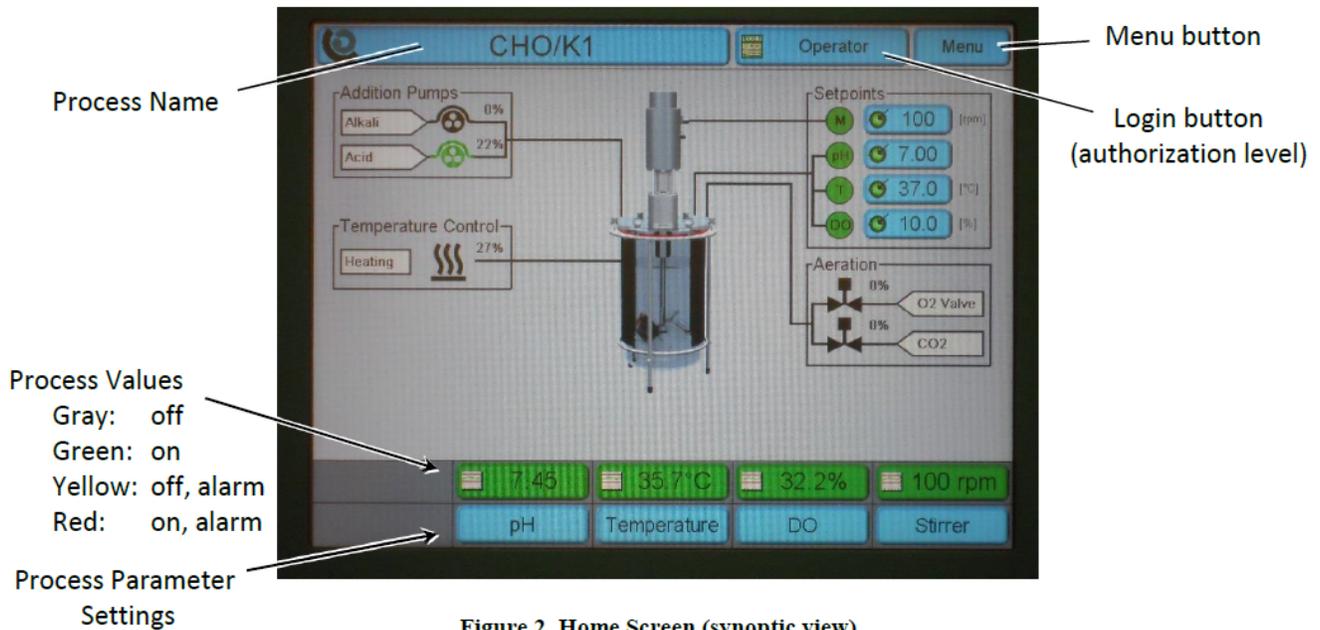


Figure 2. Home Screen (synoptic view)

Example 1. Navigating the Controller Screens to Login

Instructions	Explanation
Home > Login (top middle) > Operator > Login Enter the Operator password: "0000"	On the Home screen, touch the Login button (top middle). The display changes to the Access Control screen. On the Access Control screen, touch the button Operator (left side). The display changes to the Operator screen. On the Operator screen, touch the button Login. The display changes to a keyboard and prompts for the Operator password. Enter the Operator password: "0000" Then touch the button Enter. The display changes back to the Home screen.

SOP: Applikon EZ-Control Bioreactor Controller Operation

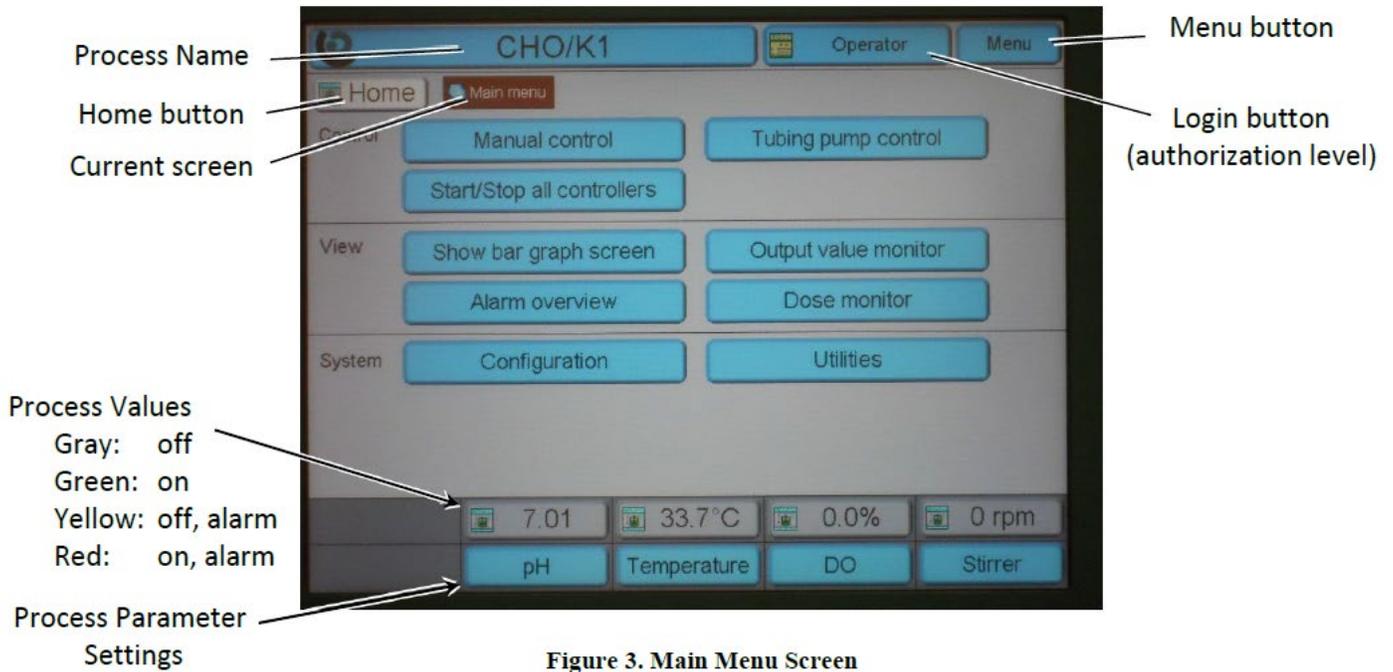


Figure 3. Main Menu Screen

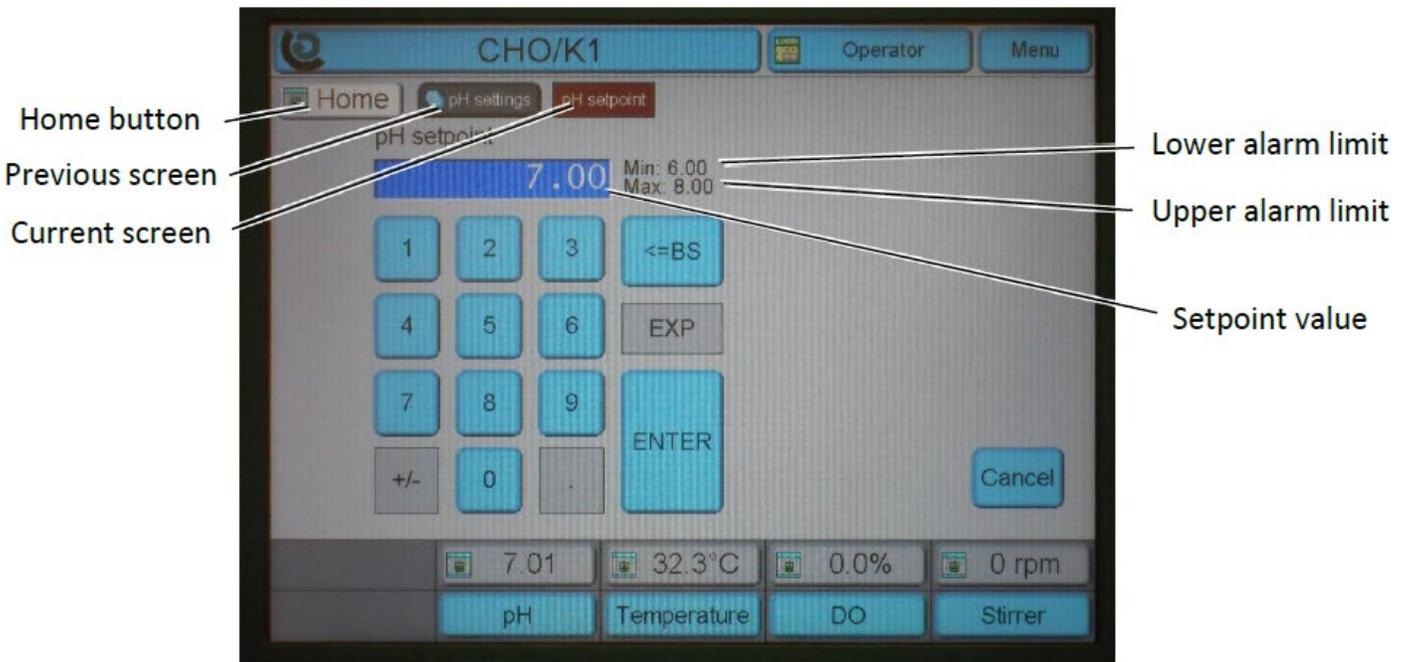


Figure 4. pH Setpoint Screen

SOP: Applikon EZ-Control Bioreactor Controller Operation

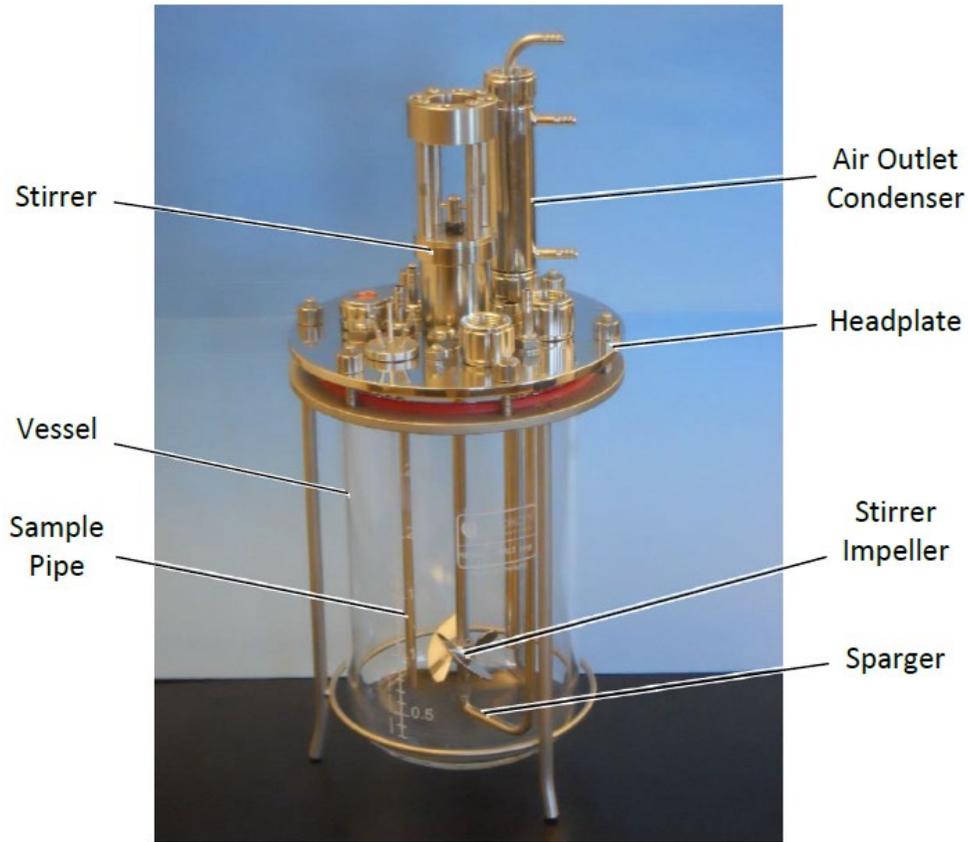


Figure 5. Applikon 3-Liter Glass Autoclavable Bioreactor

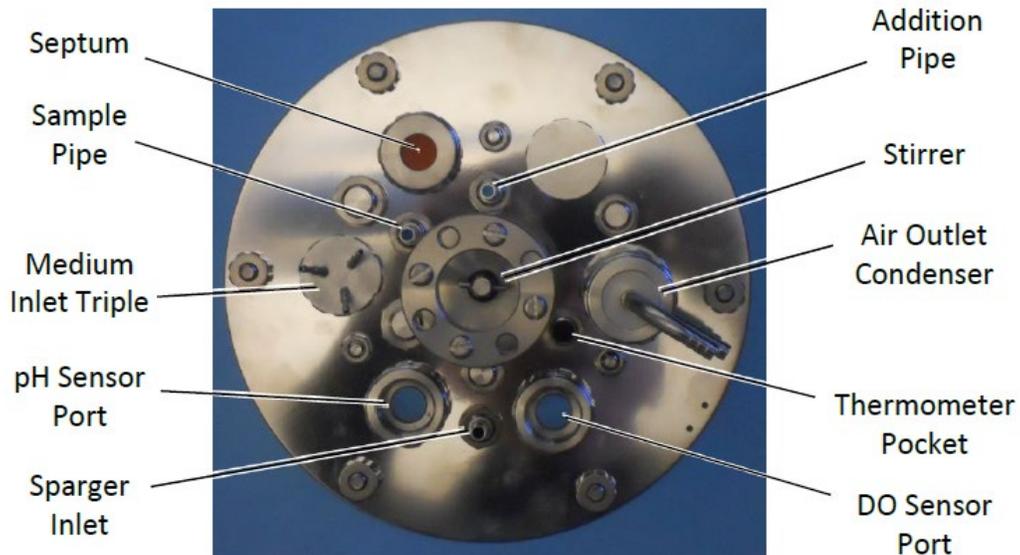


Figure 6. Bioreactor Head Plate (top view, unassembled)

SOP: Applikon EZ-Control Bioreactor Controller Operation



Figure 7. Liquid Addition Bottle

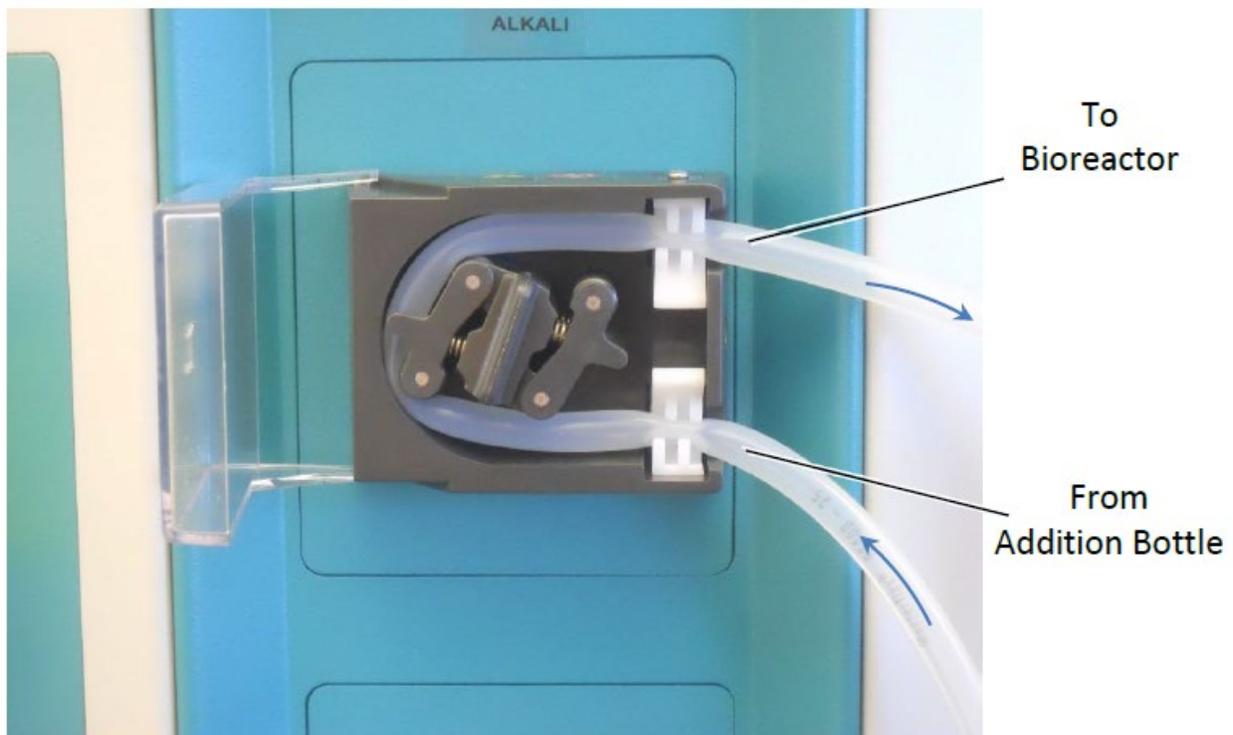


Figure 8. Peristaltic Pump (Alkaline Pump)

SOP: Applikon EZ-Control Bioreactor Controller Operation

10. History

<i>Revision Number</i>	<i>Effective Date</i>	<i>Preparer</i>	<i>Description of Change</i>
0	20DEC13	John Buford	Initial Release
1	16SEP14	Jason McMillan	Various fixes and adjustments
2	10JUL17	Hetal Doshi	Added Details

Quality Control Biochemistry: Upstream Processing

SOP: Trypan Blue Assay for Cell Viability Determination

Approvals:

Preparer: Jason McMillan

Date: 02JAN14

Reviewer: Hetal Doshi

Date: 20DEC18

Reviewer: Dr. Maggie Bryans

Date: 20DEC18

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 50 μ L of culture sample and dispense into a microfuge tube.

8.1.3. Add 50 μ L of 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.

SOP: Trypan Blue Assay for Cell Viability Determination

8.2. Transfer sample to hemacytometer

- 8.2.1. Center the coverslip on top of the hemacytometer. The metal notches on a hemacytometer should be partially exposed.
- 8.2.2. Hold the micropipette straight up and dispense 15 μ L of the cell/Trypan Blue solution into a metal notch of the hemacytometer. The tip of the pipette should be very close to the metal surface. The solution will spread through capillary action.
- 8.2.3. Repeat step 8.2.2 for the second grid.

8.3. Observe cells under the microscope

- 8.3.1. Turn on the microscope per SOP.
- 8.3.2. Place the hemacytometer on the microscope stage.
- 8.3.3. Focus on the hemacytometer grid using 100X magnification (10X objective lens). Live cells are clear. Dead cells are blue.

8.4. Count cells

- 8.4.1. The grid is divided into four main quadrants (Figure 2). Beginning with quadrant 1 and moving through to quadrant 4, depress the correct button on the push button counter for every cell in each square.
 - 8.4.1.1. Make sure the counter is set to 0. Count total live cells first. After live cell count is completed, count total dead cells.
 - 8.4.1.2. Count in a serpentine fashion: work left to right across the top row of the quadrant. Move down to the second row and count the cells in each square moving right to left. Change to opposite direction each time a row is completed.
 - 8.4.1.3. Count cells touching the top and left borders of a main quadrant, but not the bottom and right borders. **Do not count cells outside of the main quadrants.**
 - 8.4.1.4. Record the number of live and dead cells each time a quadrant is completed.
- 8.4.2. Repeat counting procedure per section 8.4.1. for the second grid.
- 8.4.3. Average the cell counts of the two grids to obtain the live cell count for the viable cell concentration calculations.

8.5. Clean the hemacytometer

- 8.5.1. Remove the coverslip.
- 8.5.2. Blot dry the coverslip and hemacytometer on a lab towel.
- 8.5.3. Rinse the coverslip and hemacytometer with 70% EtOH by holding each one over a lab towel and using a squirt bottle containing 70% EtOH. Repeat rinsing with milliQ H₂O and use squirt bottle labelled milliQ H₂O. **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: $C = (N/V) \times D$
C = live cell count in cells per milliliter
N = total number of live cells obtained from averaging the number of live cells

SOP: Trypan Blue Assay for Cell Viability Determination

counted in the two grids

V = volume of counting area

Note: The total volume of the four quadrants is 0.0004mL. (Each quadrant is 0.0001mL.)

D = dilution factor. For this procedure the dilution factor is 2.

8.7. Calculate percent viability

8.7.1. Formula for percent viability: **% viability = (live cell count/total cell count) *100**

9. Attachments:

9.1. Figure 1: Diagram of hemacytometer and cover glass

9.2. Figure 2: Diagram of hemacytometer quadrants

10. History:

Name	Date	Amendment
Jason McMillan	02JAN14	Initial Release
Hetal Doshi	20DEC18	Change the sample and trypan blue volume to 50µl and sample loading volume to 15µl

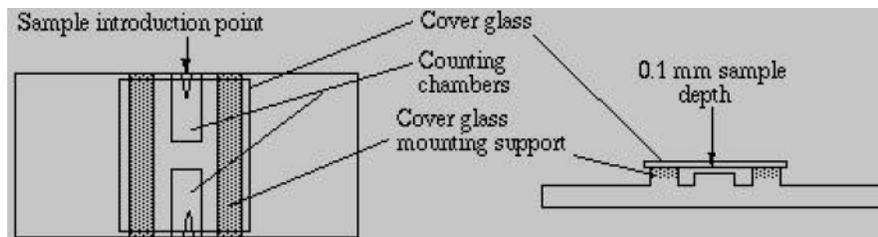


Figure 1: Diagram of hemacytometer and cover glass

Image: <http://plaza.ufl.edu/johnaris/Protocols/MiscMethods/HemoCytometer.pdf>

SOP: Trypan Blue Assay for Cell Viability Determination

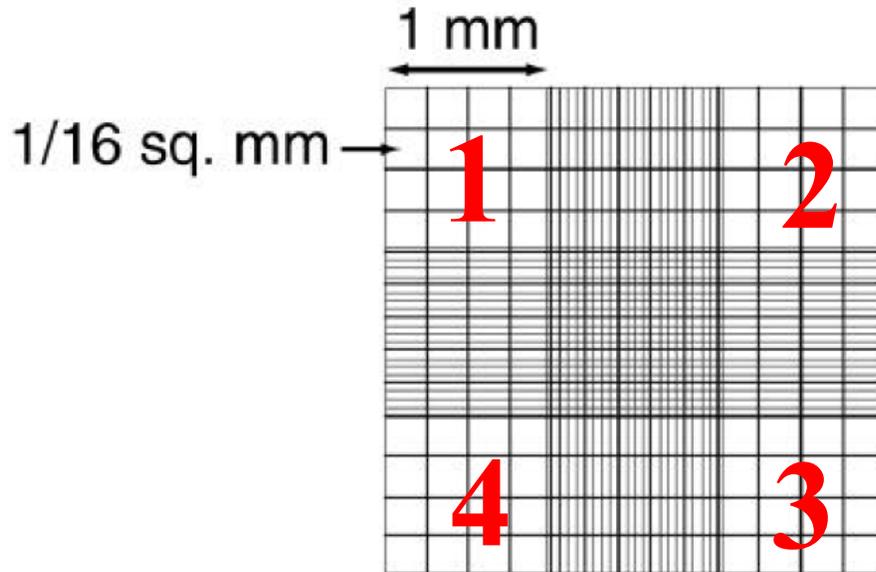


Figure 2: Diagram of hemacytometer quadrants

SOP: Glucose Determination Assay Using Spectrophotometry

Approvals:

Preparer: Jason McMillan

Date: 26JUN14

Reviewer: Dr. Maggie Bryans

Date: 27JUN14

1. Purpose:

1.1. Use of the Glucose Determination Assay.

2. Scope:

2.1. Applies to the quantitative determination of Glucose in Conditioned Media.

3. Responsibilities:

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

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

4. References:

4.1. <http://www.pointscientific.medtest.com/uploads/inserts/G7521-01-934.pdf>

5. Precautions:

5.1. The reagent should not be used if it has developed turbidity or other signs of microbial growth.

5.2. The reagent should not be used if it fails to meet linearity claims or fails to recover control values in the stated range.

6. Materials:

6.1. P-20 and P-1000 micropipette and tips

6.2. Micro centrifuge tubes

6.3. Timer

6.4. Spectrophotometer able to read at 500nm

6.5. Cuvettes

6.6. Water bath (37°)

6.7. Glucose reagent

6.8. Glucose standard

6.9. Control with known normal range

7. Procedure:

7.1. Running Assay

7.1.1. Turn on water bath and set to 37°C.

7.1.2. Label micro centrifuge tubes “Blank,” Control,” Standard,” “Sample Name #'s.”

7.1.3. Pipette 1.0ml of working reagent to all of the tubes and place in the 37°C water bath for 5 minutes.

7.1.4. Remove micro centrifuge tubes from the water bath.

7.1.5. Add 10µl of control solution to the “Control” tube, 10µl of Glucose standard to the “Standard” tube, and 10µl of sample to each of their respective “Sample” micro centrifuge tubes and mix by gently aspirating and dispensing the solution with the micropipette.

7.1.6. Place all of the micro centrifuge tubes except for the “Blank” micro centrifuge tube back into the 37°C water bath for 10 minutes.

SOP: Glucose Determination Assay Using Spectrophotometry

- 7.1.7. Remove the micro centrifuge tubes from the 37°C water bath and immediately remove 1ml from each microfuge tube and place it in a corresponding labeled cuvette.
- 7.1.8. Read and record the absorbance of the tubes at 500nm using the “Blank” tube to zero the spectrophotometer.
- 7.1.9. Record absorbance values for each of the tubes and calculate the concentration of Glucose.

7.2. Calculate Glucose Concentration.

- 7.2.1. Formula to determine glucose concentration:

$$\text{Glucose (mg/dl)} = \frac{\text{Abs of sample}}{\text{Abs of standard}} \times \text{Concentration of standard (mg/dl)}$$

8. History:

Name	Date	Amendment
Jason McMillan	26JUN14	Initial release

SOP: Lactate Determination Assay Using Spectrophotometry

Approvals:

Preparer: Jason McMillan

Date: 26JUN14

Reviewer: Dr. Maggie Bryans

Date: 27JUN14

1. Purpose:

1.1. Use of the Lactate Determination Assay.

2. Scope:

2.1. Applies to the quantitative determination of Lactate in Conditioned Media.

3. Responsibilities:

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

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

4. References:

4.1. <http://www.pointscientific.medtest.com/uploads/inserts/L7596-01-984.pdf>

5. Precautions:

5.1. Reagents contain sodium azide as preservative. Upon disposal flush with large volumes of water.

5.2. Do not use the reagents beyond the expiration date printed on the label.

6. Materials:

6.1. P-20 and P-1000 micropipette and tips

6.2. Micro centrifuge tubes

6.3. Timer

6.4. Spectrophotometer able to read at 550nm

6.5. Cuvettes

6.6. Heating block (37°)

6.7. Lactate reagents R1 and R2

6.8. Lactate standard

6.9. Control with known normal range

7. Procedure:

7.1. Running Assay

7.1.1. Turn on heating block and set to 37°C.

7.1.2. Label micro centrifuge tubes "Blank," "Control," "Standard," "Sample Name #'s."

7.1.3. Prepare Lactate working reagent by combining R1 and R2 using a 3 to 2 ratio. Ex: Mix 3ml of R1 reagent with 2ml of R2 reagent.

7.1.4. Pipette 1.0ml of working reagent to all of the tubes and place in the 37°C heating block for 5 minutes.

7.1.5. Remove micro centrifuge tubes from the heating block.

7.1.6. Add 10µl of control solution to the "Control" tube, 10µl of Lactate standard to the "Standard" tube, and 10µl of sample to each of their respective "Sample" micro centrifuge tubes and mix by gently aspirating and dispensing the solution with the micropipette.

7.1.7. Place all of the micro centrifuge tubes except for the "Blank" micro centrifuge tube back into the 37°C heating block for 5 minutes.

SOP: Lactate Determination Assay Using Spectrophotometry

- 7.1.8. Remove the micro centrifuge tubes from the 37°C heating block and immediately remove 1ml from each microfuge tube and place it in a corresponding labeled cuvette.
- 7.1.9. Read and record the absorbance of the tubes at 550nm using the “Blank” tube to zero the spectrophotometer.
- 7.1.10. Record absorbance values for each of the tubes and calculate the concentration of Lactate.
- 7.2. **Calculate Lactate Concentration.**
 - 7.2.1. Formula to determine lactate concentration:
$$\text{Lactate (mmol/L)} = \frac{\text{Abs of sample}}{\text{Abs of standard}} \times \text{Concentration of standard (mmol/L)}$$
 - 7.2.2. If the result exceeds 20 mmol/L, the sample should be diluted 1:1 with normal saline, ran again, and the result multiplied by 2.

8. History:

Name	Date	Amendment
Jason McMillan	26JUN14	Initial release

Glucose and Lactate Concentration Determination using the YSI 2900 Biochemistry Analyzer

Approvals

Prepared by: Cianna Cooper

Date: 24 March 2014

Reviewed by: Dr. Linda Rehfuss

Date: 02 April 2014

1. Purpose:

1.1. The operation of the YSI 2900 Biochemistry Analyzer to measure the glucose and lactate concentrations in samples. The YSI 2900 Analyzer utilizes enzyme sensor technology to measure concentrations of up to two analytes in solution. Using this technology, enzymes are immobilized in a 3-layer membrane on platinum probes in the instrument. When samples containing glucose or lactate are injected on to the membranes, they are oxidized to hydrogen peroxide (H₂O₂). Hydrogen peroxide (H₂O₂) is then oxidized at the platinum probe and produces a probe current. The probe current at each probe is proportional to the amount of hydrogen peroxide and therefore to the amount of glucose or lactate in the sample. This SOP describes the use of the YSI 2900 to measure glucose and lactose concentration in samples.

2. Scope:

2.1. This SOP applies to operation of the YSI 2900 Biochemistry Analyzer to measure the glucose and lactate concentrations in samples by students in the Biomanufacturing class (BIOT 221) in the STEM Department of Bucks County Community College

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 YSI 2900 Operations Manual

5. Definitions: Not Applicable

6. Precautions:

6.1 Wear personal protection equipment (laboratory coat and gloves) when operating the instrument and handling samples.

7. Materials:

- 7.1 YSI Buffer Kit (YSI # 2357)
- 7.2 YSI Glucose Membrane Kit (YSI # 2365)
- 7.3 YSI Lactate Membrane Kit (YSI # 2329)
- 7.4 YSI Glucose-/Lactate Standard (YSI # 2747- glucose 1.80 g/L, L-lactate-0.45 g/L)

Glucose and Lactate Concentration Determination using the YSI 2900 Biochemistry Analyzer

8. Procedure:

8.1. Prime the instrument fluid system

- 8.1.1. From the configure screen , touch the “initialize” tab
- 8.1.2 Touch the “prime” button under bottle B1 to prime the buffer solution
- 8.1.3 Touch the “prime” button under Bottle C1 to prime the calibrator bottle

8.2 Check Probe Current

- 8.2.1 From the “initialize” tab of the Configure screen, touch either “flush” button to flush the sample chamber with buffer
- 8.2.2 Observe the probe current values. They must be below 6 nA and stable. When the enzyme probe baseline currents are below 6 nA and stable, the probe indicators change from red to green. Once both probe indicators are green, touch the “X” button at the top left of the screen to exit the main display

8.3. Calibration- The instrument must be calibrated before running samples

- 8.3.1 From the main display, touch “Run” From the Calibrate tab, Touch “start” to initiate calibration. The instrument will initiate calibration of active probes. Calibration status will be displayed on the screen.

8.4 Read Samples

- 8.4.1 From the Run screen, touch the Sample tab.
- 8.4.2 Touch the “Station 2” icon. The station 2 set-up window appears.
- 8.4.3 Touch the “manual” button to change sample name. When the keypad window appears, enter a new sample name by touching the buttons. Touch done and the new sample name will appear.
- 8.4.4 Hold sample tube at Station 2 and touch “start” to initiate sampling at Station 2. results will be displayed. Record results.

9. Attachments: N/A

10. History:

Revision	Reviewer	Date	Revision Number
Initial Release	Dr. Linda Rehfuss	09 April 2014	0

SOP: Quantification of CHO DP-12 Derived Anti IL-8 Monoclonal Antibody by ELISA

Approvals

Preparer: Robin Zuck

Date: 18 Jan 2017

Reviewer: Hetal Doshi

Date: 13Jan 2018

Reviewer: Dr. Margaret Bryans

Date: 13Jan 2018

1. Purpose

- 1.1. Quantitative determination of the concentration and/or titer of CHO-DP12 derived Humanized Mouse anti-Human IL-8 monoclonal antibodies.

2. Scope and Applicability

- 2.1. This ELISA Assay may be used for quantitative determination of Humanized Mouse anti-Human IL-8 monoclonal antibodies in cell culture media and chromatography buffers. This assay can be run using a standard curve to calculate antibody concentration or without a standard curve to determine antibody titer.

3. Summary of Method

- 3.1. Coat 96 well plate with human IL-8
- 3.2. Block coated 96 well plate
- 3.3. Preparation of standard
- 3.4. Preparation of test sample dilutions
- 3.5. Standard and test sample addition and incubation
- 3.6. Secondary antibody addition and incubation
- 3.7. Substrate incubation
- 3.8. Addition of Stop solution
- 3.9. Measurement
- 3.10. Calculation of results

4. References

- 4.1. Antibodies a Laboratory Manual; Ed Harlow, David Lane, Publisher: Cold Spring Harbor Laboratory Press
- 4.2. ELISA Handbook <https://www.bosterbio.com/ebooks>
- 4.3. Bio Rad iMark Microplate Absorbance Reader SOP

5. Precautions

- 5.1. None

6. Responsibilities

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

SOP: Quantification of CHO DP-12 Derived Anti IL-8 Monoclonal Antibody by ELISA

7. Equipment and Materials

- 7.1. Fisher brand polystyrene 96 well plate, catalog #12565501 or equivalent.
- 7.2. Bovine Serum Albumin, (BSA), Fisher catalog #BP9703-100, or equivalent.
- 7.3. 1X PBS, Hyclone catalog # SH30256.01 or equivalent.
- 7.4. Human IL-8, without BSA or other protein stabilizer.
- 7.5. Rabbit anti-Human IgG peroxidase conjugated antibody, ThermoFisher catalog # PA1-28587, or equivalent.
- 7.6. TMB, Invitrogen REF # SB01, or equivalent.
- 7.7. 20 μ l, 200 μ l, 1000 μ l pipettes and tips
- 7.8. A 30-300 μ l multichannel pipette and tips, if available.
- 7.9. Shaking platform capable of reaching 300rpm.
- 7.10. Bio Rad iMark Microplate Absorbance Reader.
- 7.11. Microtubes and rack
- 7.12. Blocking Buffer (3% BSA (w/v) in 1X PBS buffer (pH 7.4))
- 7.13. 1N HCl
- 7.14. Purified anti IL-8 mAb to use as a standard
- 7.15. Anti-Human IL-8 antibody Test Samples from Spinner Flask, Bioreactor and Chromatography steps

8. Procedure

- 8.1. Coat the number of required wells of a polystyrene 96 well plate with Human IL-8 at 800ng/well.
 - 8.1.1. Prepare enough of an 8ng/ μ l IL-8 in 1X PBS coating solution for the required number of wells, use 100 μ l for each well. To coat 20 wells in a 96 well plate prepare 2,000 μ l, (16 μ l of a 1mg/ml IL-8 stock and 1,984 μ l of 1X PBS).
 - 8.1.2. Pipette 100 μ l of the coating solution into each well of the 96 well plate.
 - 8.1.3. Seal the plate and incubate overnight at 4°C on a shaking platform capable of reaching 300rpm.
 - 8.1.4. Invert the plate over the sink to remove the coating solution and then tap on paper towels to be sure all the coating solution is removed.
 - 8.1.5. Wash the plate twice using 250 μ l of 1X PBS per well, invert the plate and tap on paper towels as above to remove the wash buffer from the wells.
- 8.2. Block the coated wells of the 96 well plate.
 - 8.2.1. Pipette 250 μ l of Blocking Buffer, (3% w/v BSA in 1X PBS), into each of the coated wells of the plate.
 - 8.2.2. Seal the plate and incubate for 2 hours at room temperature or overnight at 4°C, on a shaking platform capable of reaching 300rpm.
- 8.3. Preparation of Standard and Test Sample solutions; (Dilutions for the standard curve and samples should be prepared before removing the blocking solution.)

SOP: Quantification of CHO DP-12 Derived Anti IL-8 Monoclonal Antibody by ELISA

- 8.3.1. From a 1ug/ml anti IL-8 mAb standard solution prepare 500ul of a 120ng/ml, 100ng/ml, 80ng/ml, 60ng/ml, 40ng/ml, 20ng/ml and 10ng/ml solutions in Blocking Buffer.
- 8.3.2. Follow the dilution table located in attachments section for standard preparation.
- 8.4. Prepare 300µl of an appropriate dilution in blocking buffer of each test sample (typically 1:10, 1:100 or 1:1000 dilutions are used).
- 8.5. Uncover the coated and blocked microtiter plate and remove the blocking solution by inverting the plate over the sink and then tap on paper towels. Wash the plate 3times with 1X PBS as above.
- 8.6. Add 100µl of anti-IL-8 mAb standards in duplicate wells and test samples in single wells and cover the plate. Be sure to carefully note their position on the microtiter plate and record in SOP plate template.
 - 8.6.1. Shake plate at 300rpm for 1 hour at room temperature.
 - 8.6.2. Wash the plate three times as above with 1X PBS.
- 8.7. Secondary Antibody Addition (Rabbit anti-Human IgG).
 - 8.7.1. Prepare a 1:16,000 dilution of the Rabbit anti-Human IgG antibody in blocking buffer.
 - 8.7.2. Add 100µl of secondary antibody to all wells and cover the plate.
 - 8.7.3. Shake plate at 300rpm for 1 hour at room temperature.
 - 8.7.4. Wash wells three times as above with 1X PBS.
- 8.8. Substrate Incubation
 - 8.8.1. Add 100µl of TMB substrate to all wells and shake at 300 rpm for approximately 3-6 minutes, until a color gradient is observed in the standard wells, (usually 3-6 minutes). See Figure 3.
 - 8.8.2. Stop the reaction by adding 100µl of 1N HCl in the same order as the substrate was added to the wells. The color will change from blue to yellow. Mix thoroughly by gently tapping the microtiter plate. The color in each well should appear uniform.
- 8.9. Measurement
 - 8.9.1. Measure the absorbance in all wells at 450nm using the Bio Rad iMark Microplate Absorbance Reader.
- 8.10. Calculation of Results
 - 8.10.1. Subtract the value of the zero-point standard from all of the standards and unknowns to determine the corrected absorbance (A_{450nm}).
 - 8.10.2. Calculate the average of the duplicate standard wells.
 - 8.10.3. Plot A_{450nm} against the concentration of anti-IL-8 mAb in the standards, to create an anti IL-8 mAb standard curve.
 - 8.10.4. Use a linear fit to plot the points of the anti-IL-8 mAb standard curve.
 - 8.10.5. The amount of anti IL-8 mAb in the unknowns can be determined from the slope of the standard curve.
 - 8.10.6. For upstream processing, create a graph showing the concentration of anti-IL-8 mAb of the Culture in days.

SOP: Quantification of CHO DP-12 Derived Anti IL-8 Monoclonal Antibody by ELISA

8.10.7. For downstream processing, determine the concentration of anti IL-8 mAb in pre and post Protein A chromatography samples.

Note: The 120ng/ml point may not fall within the linear portion of the curve. If this point is not in the linear portion of the curve remove it from your standard curve.

9. Attachments

Anti IL-8 mAb Concentration (ng/ml)	Dilutions
0	100µl/well (blocking buffer) Zero point to determine background
10	
20	
40	
60	
80	
100	
120	

Figure 1. Dilution table for preparation of anti IL-8mAb standard

SOP: Quantification of CHO DP-12 Derived Anti IL-8 Monoclonal Antibody by ELISA

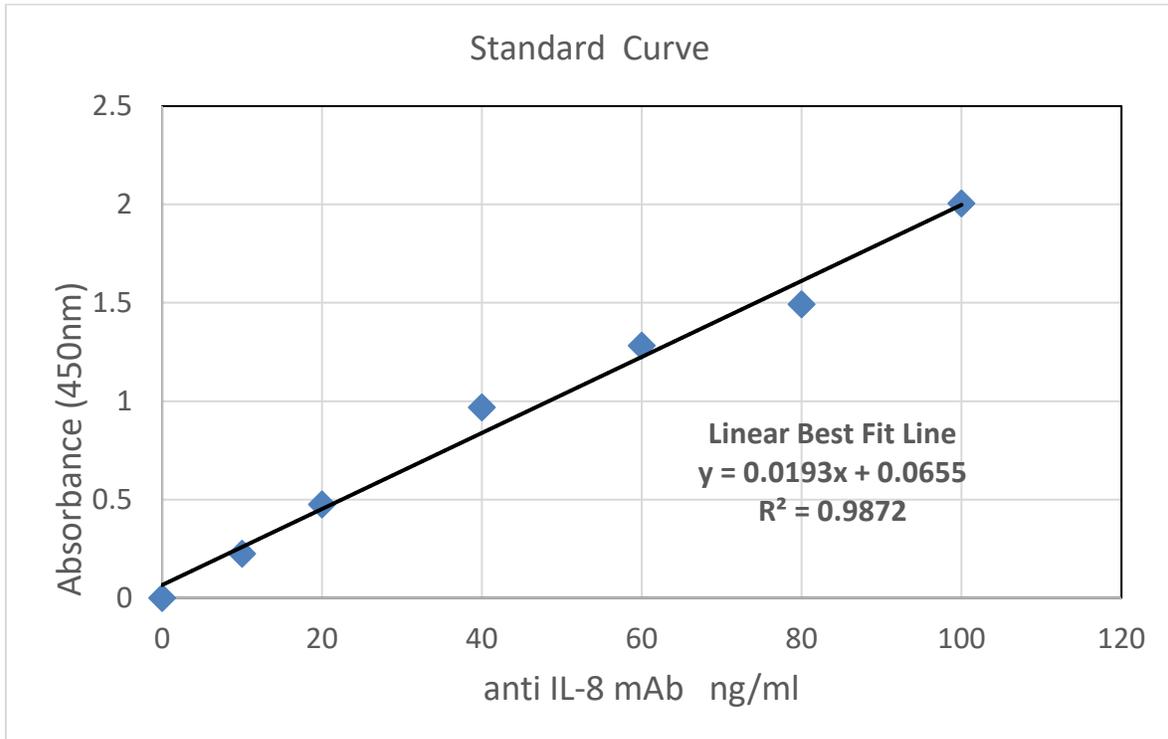


Figure 2. Anti IL-8 mAb standard curve (Example Only)

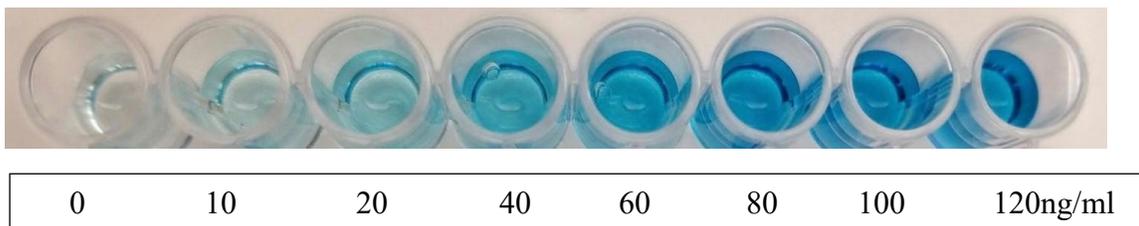


Figure 3. Anti IL-8 mAb standard curve wells before addition of Stop solution.

SOP: Quantification of CHO DP-12 Derived Anti IL-8 Monoclonal Antibody by ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Figure 5. ELISA Plate Layout

10. History

<i>Revision Number</i>	<i>Effective Date</i>	<i>Preparer</i>	<i>Description of Change</i>
0	07JUL2017	Robin Zuck	Initial release
1	13JAN2018	Robin Zuck	Change secondary antibody to Rb anti Human IgG Edited title to include "Humanized Mouse Anti IL-8 Antibody"

SOP: Operation of Bio Rad iMark Microplate Absorbance Reader

Approvals

Preparer: Jason McMillan

Date: 09APR14

Reviewer: Dr. Margaret Bryans

Date: 09APR14

1. Purpose

1.1. To measure absorbance values for use in determining protein concentration and activity.

2. Scope and Applicability

2.1. The Bio Rad iMark Microplate Absorbance Reader is an eight-channel, vertical path length photometer that measures the absorbance of the contents in the wells of a 96-well microtitration plates. It can perform single or dual wavelength measurements and can report absorbance values to three decimal places.

3. Summary of Method

3.1. Turn on and load 96 well plate into Bio Rad iMark Microplate Absorbance Reader.

3.2. Run protocol.

3.3. Remove 96 well plate and turn off Bio Rad iMark Microplate Absorbance Reader

4. References

4.1. iMark Microplate Absorbance Reader Instruction Manual

5. Precautions

5.1. Be sure to open and close the “Reading Chamber Door” by pressing the “Open/Close” key on the “Keypad”. Attempting to manually open or close the “Reading Chamber Door” can lead to damage.

6. Responsibilities

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

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

7. Equipment and Materials

7.1. iMark Microplate Absorbance Reader

7.2. 96 Well Plate

8. Procedure

8.1. Turn on the iMark Microplate Absorbance Reader by holding the “Power Button” until the “LCD” illuminates

8.1.1. The “LCD” will display “Self Diagnosis” followed by “Initializing” Once initialization is complete, a login screen will appear.

8.1.2. The login name will read “Common User” and a prompt for a password will be shown. Enter “00000” and press “Enter” on the “Keypad.”

8.1.3. “Lab Name, Kit Name, Reading Mode, and Measurement Filter” appears on screen (be sure to select Measurement Filter specific for wavelength), self-initialization is complete, and the Bio Rad iMark Microplate Absorbance Reader is ready for measurement.

8.2. Open the “Reading Chamber Door” by pressing the “Open/Close” key on the “Keypad”.

SOP: Operation of Bio Rad iMark Microplate Absorbance Reader

- 8.2.1. Once “Reading Chamber Door” is open, place 96 Well Plate into the “Reading Chamber” being sure to line the 96 Well Plate with the plate holding guides.
 - 8.3. Close the “Reading Chamber Door” by pressing the “Open/Close” key on the “Keypad”.
 - 8.4. Start measurement reading by pressing the “Start/Stop” key on the “Keypad”. The measurement function will begin and all data will be printed indicating the completion of the measurement function.
 - 8.4.1. Remove data printout
 - 8.5. Open the “Reading Chamber Door” by pressing the “Open/Close” key on the “Keypad”.
 - 8.5.1. Once “Reading Chamber Door” is open, remove 96 Well Plate from the “Reading Chamber.”
 - 8.6. Close the “Reading Chamber Door” by pressing the “Open/Close” key on the “Keypad”.
 - 8.7. Turn off the Bio Rad iMark Microplate Absorbance Reader by holding the “Power Button” until the “LCD” reads “Power Off” and “Yes” is highlighted, press “Enter” key. Bio Rad iMark Microplate Absorbance Reader will shut down.
- ### 9. Attachments



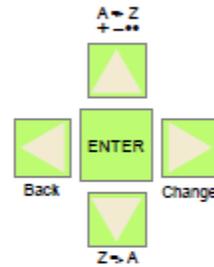
Figure 1. External Features of Bio Rad iMark Microplate Absorbance Reader

SOP: Operation of Bio Rad iMark Microplate Absorbance Reader

Main	Returns to MAIN SCREEN.
Start/Stop	Initiates plate reading using current active protocol. Stops plate reading and printing.
Paper Feed	Advances paper strip in internal printer.
Print	Prints out the plate data and protocol information.
Edit	Enters the Edit Menu, and sets up the instrument.
Memory Recall	Reads out protocol and plate data.
Open/Close	Opens or closes the reading chamber door.



Up Arrow	Moves the cursor upward. Selects the alphabet or the symbol.
Left Arrow	Returns to the previous screen. Moves the cursor to the left.
Down Arrow	Moves the cursor downward. Selects the alphabet or the symbol.
Right Arrow	Moves the cursor to the right. Changes or selects the value and type.
Enter	Completes or seals a field entry.



Dot/Function	Inputs the dot. Changes the input mode.
Ten Keys	Inputs numbers or well type in plate mapping. 0 / EMP : Empty 5 / QC : QC control 1 / SMP : Sample 6 / CAL : Calibrator 2 / BLK : Blank 7 / CP : Positive Control 3 / STD : Standard 8 / CN : Negative Control 4 / CO : Cutoff control 9 / CW : Weak Positive Control



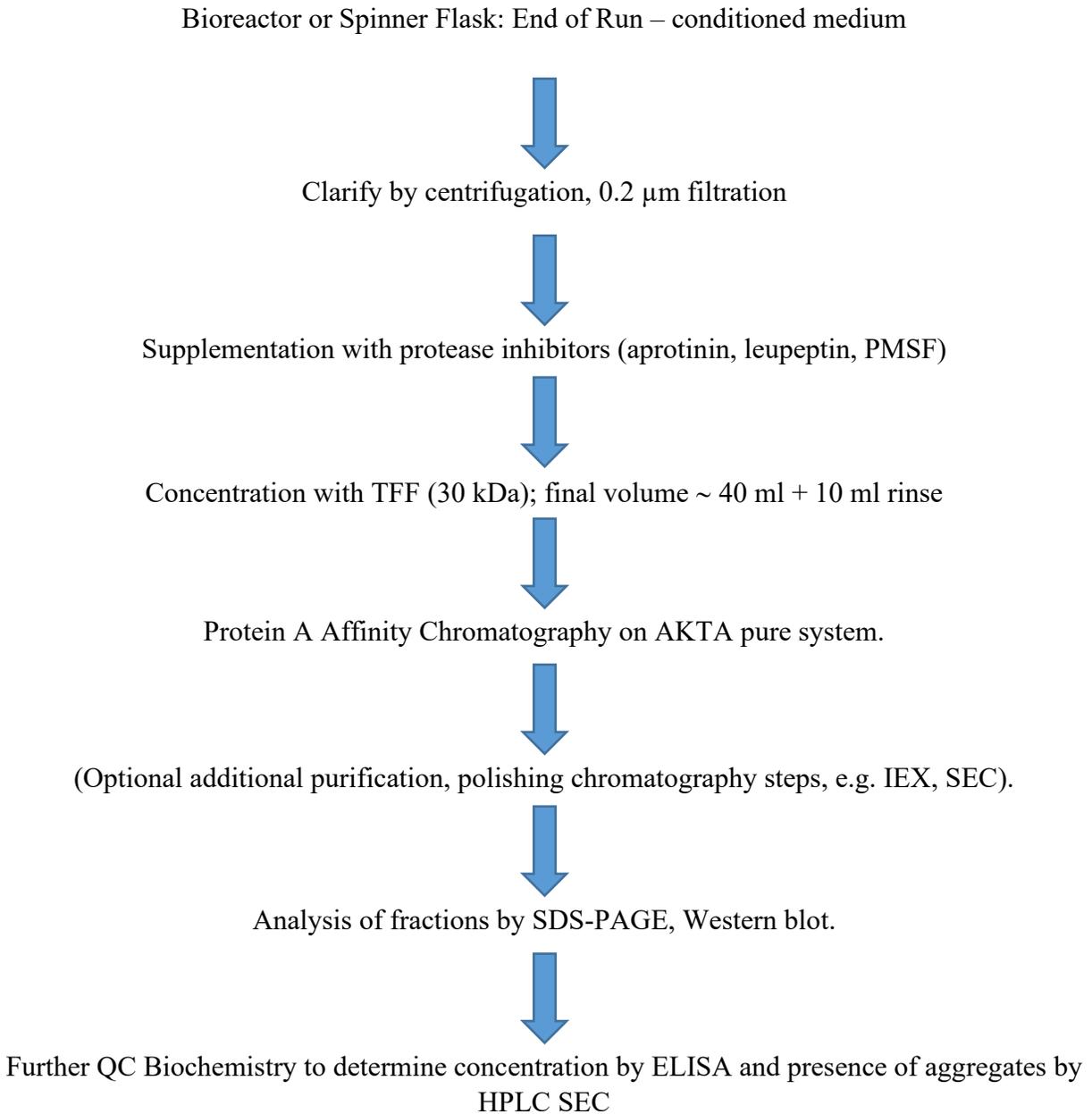
Figure 2. Keypad Guide for Bio Rad iMark Microplate Absorbance Reader

10. History

<i>Revision Number</i>	<i>Effective Date</i>	<i>Preparer</i>	<i>Description of Change</i>
0	09APR14	Jason McMillan	Initial release

Downstream Processing: Purification of Anti IL-8 mAb

Flow Chart: mAb Downstream Process



SOP: End-of-Run Anti IL-8 mAb Process: Harvest, Centrifugation, TFF Concentration

Approvals:

Preparer: Hetal Doshi

Date: 25MAR20

Reviewer: Dr. Maggie Bryans

Date: 25MAR20

1. Purpose:

- 1.1. To harvest anti IL-8 mAb containing conditioned medium, concentrate and prepare for chromatography; intermediate steps include centrifugation and sterile filtration to remove cells and cell debris prior to concentrating and buffer exchange by tangential flow filtration.

2. Scope and Applicability:

- 2.1. A biomanufacturing environment requires proper steps to recover and purify active pharmaceutical ingredient from a bioreactor or fermentor. This SOP provides bench scale procedures to accomplish that goal using conditioned medium from cells expressing recombinant anti IL-8 mAb. The method demonstrates the principles of tangential flow filtration, centrifugation, and sterile filtration in preparation for downstream processing by column chromatography as may be employed in a typical process development, for later scale up to manufacturing.

3. Summary of Method:

- 3.1. Preparation of solutions:
 - 3.1.1. PBS/Tween 80 for preconditioning of the Pellicon cassette (for TFF)
 - 3.1.2. 0.1 N NaOH for cleaning the Pellicon cassette following use
 - 3.1.3. 0.05 N NaOH for storage of the Pellicon cassette
- 3.2. Flushing and preconditioning of TFF/Pellicon.
- 3.3. Transfer of culture from bioreactor to centrifuge bottles.
- 3.4. Centrifugation to pellet cells.
- 3.5. Sterile filtration of the conditioned medium(CM) using 0.22 micron vacuum filter units with storage bottle.
- 3.6. Addition of protease inhibitors and Tween 80.
- 3.7. Concentration of supplemented CM by tangential flow filtration.

4. References:

- 4.1 Millipore Tangential Flow and Diafiltration Using Pellicon XL Device of tPA SOP
- 4.2 Oakton PC 700 Bench Series pH/Conductivity/°C/°F Meter SOP (Doc # 1.0).

5. Definitions:

- 5.1. Permeate- the material that passes through the membrane.
- 5.2. Retentate- the material that does not pass through the membrane.
- 5.3. TFF – tangential flow filtration
- 5.4. CM – conditioned medium, which contains the API of interest

SOP: End-of-Run Anti IL-8 mAb Process: Harvest, Centrifugation, TFF Concentration

6. Precautions:

- 6.1. 0.1N NaOH is very corrosive. It is extremely damaging to eyes and mucous membranes. It causes burns. Avoid contact with skin. It is harmful if swallowed or inhaled. The Millipore Pellicon XL Device is stored flat at 4-25°C with 10 mL of 0.1N NaOH.
- 6.2. NEVER tighten the clamp enough to completely restrict the flow in the Retentate tube. This could damage the filter and cause the tubing to disconnect.
- 6.3. Luer Lock fittings on the TFF device should be tightened with care not to exert too much force, to avoid stripping threads or damaging the fitting.

7. Responsibilities:

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

8. Equipment and Materials:

- 8.1. 250 mL Nalgene centrifuge bottles (3)
- 8.2. 250 mL Corning bottles (3)
- 8.3. 0.1N NaOH (sodium hydroxide)
- 8.4. 0.05N NaOH (sodium hydroxide)
- 8.5. 10% (w/v) Tween 80
- 8.6. Preconditioning buffer (PBS containing 0.1% Tween 80)- 50 mL
- 8.7. Stock solutions of protease inhibitors:
 - 8.7.1. 10 mg/mL PMSF (phenylmethylsulfonylfluoride); 250X
 - 8.7.2. Leupeptin, 2 mg/mL; 4000x
 - 8.7.3. Aprotinin, 10 mg/mL, 5000x
- 8.8. Millipore Tangential Flow Filtration System and Pellicon XL Device and Accessories
- 8.9. MilliQ Water
- 8.10. 10 mL graduated cylinder
- 8.11. 0.22 micron Nalgene vacuum sterile filtration unit with storage bottle (3)

9. Procedure:

- 9.1. Preparation of solutions (provided).
 - 9.1.1. Prepare 0.1N NaOH for cleaning.
 - 9.1.1.1. Using a 1L graduated cylinder, measure 625ml of MilliQ water.
 - 9.1.1.2. Transfer the MilliQ water into 800ml beaker
 - 9.1.1.3. Weigh 2.5±0.05g of NaOH.
 - 9.1.1.4. Transfer NaOH to the beaker containing MilliQ water.
 - 9.1.1.5. Add magnetic stir bar and stir to dissolve.
 - 9.1.1.6. Sterile filter the solution and label container: 0.1N NaOH, [date], [initials], [group number], Storage: room temp, Disposal: adjust to pH 7 then drain.
 - 9.1.2. Prepare 0.05N NaOH for Pellicon XL Device Storage
 - 9.1.2.1 Using a 10 mL graduated cylinder, measure 5 mL of MilliQ water
 - 9.1.2.2 Transfer MilliQ water to 25 mL beaker

SOP: End-of-Run Anti IL-8 mAb Process: Harvest, Centrifugation, TFF Concentration

- 9.1.2.3 Using a 10 mL graduated cylinder, measure 5 mL of 0.1N NaOH
- 9.1.2.4 Transfer 5 mL of 0.1N NaOH to 25 mL beaker
- 9.1.2.5 Add magnetic stir bar and stir to mix.
- 9.1.2.6 Sterile filter the solution and label container: 0.05N NaOH, [date], [initials], Storage: room temp
- 9.1.3. 10% w/v Tween 80
 - 9.1.3.1 Measure 80ml of MilliQ water and add to a 200ml beaker with magnetic stir bar
 - 9.1.3.2 Place the beaker on a balance and tare the balance when stable
 - 9.1.3.3 Pipette 10g Tween 80(polyoxyethylene soebitam monooleate) into the beaker with water.
 - 9.1.3.4 Stir until all of the Tween 80 is dissolved:this can take 30 minutes or more to complete. Carefully adjust the stir plate rpm to provide adequate mixing vigor without introducing air bubbles or frothing.
- 9.1.4. 1X PBS with 0.1% Tween 80
 - 9.1.4.1. Measure 79.2 ml 1X PBS with 100 ml graduated cylinder and transfer to 100 ml beaker with a magnetic stirrer.
 - 9.1.4.2. Measure 0.8 ml of 10% Tween 80 with 1ml serological pipette and add to the 79.2 ml 1X PBS
 - 9.1.4.3. Mix the solution by placing the beaker on the magnetic stir plate set at . low stirring speed till Tween 80 is completely dissolved.
 - 9.1.4.3. Sterile filter the solution with the 0.2 μ m filter. Store the prepared solution in a sterile bottle labeled [date],[solution name],[team name] and [Intials]
- 9.2. Labscale 500mL Reservoir Set Up
 - 9.2.1. Install Retenate tubing

Note: All tubing lengths are recommended to minimize recirculation volume. Longer lengths may be used. After prolonged storage, the tubing may absorb a small volume of water. As a result, the tubing color may change from translucent to opaque, which is normal. Air or oven drying will return the color to translucent.

 - 9.2.1.1.Cut silicone (translucent) tubing and install fittings as shown in figure 10.
 - 9.2.1.2.Remove plugs from retenate outlet (RET OUT) and retenate inlet (RET IN) ports.
 - 9.2.1.3.Insert the male luer end of the retenate tubing into the RET OUT port and the female luer end of the retenate tubing into the RET IN port. Turn fittings until snug.
 - 9.2.2. Install Permeate tubing
 - 9.2.2.1.Cut silicone (translucent) tubing and install fittings as shown in figure 12.
 - 9.2.2.2.Remove the plug from the permeate outlet port (PERM 2) and insert the male luer end of the permeate silicone (translucent) tubing into the PERM2 port. Turn fittings until snug.
 - 9.2.3. Install Tank Outlet Valve

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- 9.2.4. Remove plug from the tank outlet port (TANK OUT) and insert the female luer end of the tank outlet valve over the TANK OUT port. Turn the lock nut until snug.
- 9.2.5. Install Vent Filter (If required)
- 9.2.6. If a sterile vent is required, remove plug from the vent (VENT) port and insert the male luer end of MILLEX filter into the vent port.
- 9.2.4. Install Stir Bar
 - 9.2.4.1. If mixing is required, open reservoir cover and drop stir bar to the bottom of the reservoir.
- 9.3. Labscale Stir Base Set Up
 - 9.3.1. Power Connection
 - 9.3.1.1. Turn Stirrer and pump speed controls to the off position.
 - 9.3.1.2. Connect power cord to the power cord receptacle located at the rear of the system base.
 - 9.3.1.3. Align detent on connector with receptacle.
 - 9.3.1.4. Press connector into receptacle and turn lock ring to secure.
 - 9.3.2. Check Operation
 - 9.3.2.1. Remove the plugs from the pump inlet and pump outlet ports.
 - 9.3.2.2. Turn on the pump speed control, set to 2, and listen for pump motor.
 - 9.3.2.3. Turn off the pump speed control.
 - 9.3.2.4. Turn on the stirrer speed control and listen for the stirrer motor.
 - 9.3.2.5. Turn off the stirrer speed control.
- 9.4. Install Pellicon XL Cassette
 - 9.4.1. Remove the plugs from FEED, RET, PERM 1, and PERM 2 ports on the Pellicon XL cassette.
 - 9.4.2. Align the Pellicon XL cassette ports with Labscale 500 ml Reservoir ports being sure the PERM and RET DEVICE ports of the Pellicon XL cassette and reservoir match. Press the cassette firmly onto the reservoir ports. Turn the lock nuts until snug.
- 9.5. Flushing of TFF and cassette.

One should become familiar with the location of ports and tubing connection points as shown in the attachments at the end of this SOP prior to beginning setup.

 - 9.5.1. Set up the apparatus and confirm that all tubing connections are secure, according to the SOP (Millipore Tangential Flow and Diafiltration Using Pellicon XL Device SOP).
 - 9.5.2. Remove the 4 plugs on the Pellicon cassette and attach the Pellicon cassette to the Labscale apparatus.
 - 9.5.3. Add 500 mL MilliQ water to the reservoir and flush the cassette as described in section 9.4.4.
 - 9.5.4. Flushing the Pellicon cassette.
 - 9.5.4.1. Disconnect retentate silicone (translucent) tubing from RET IN port and place end of retentate tubing in waste collection vessel.
 - 9.5.4.2. Place end of permeate silicone (translucent) tubing into waste collection vessel. Open retentate valve by turning the counterclockwise.

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- 9.5.4.3. Remove the reservoir cover and fill reservoir with 500 mL of MilliQ water.
Remove the plug from VENT port and open tank outlet valve.
- 9.5.4.4. Turn the pump on and increase the speed until the feed pressure gauge reads 1.38 Bar (20 psi).
- 9.5.4.5. Continue pumping to the waste collection vessel until the level in the reservoir drops to 350 mL and then turn the pump off.
- 9.5.4.6. Reconnect the retentate silicone (translucent) tubing to the RET IN port and turn the pump on. Slowly increase the pump speed until feed pressure gauge reads 1.38 Bar (20 psi). Check the system for leaks and tighten connections if leaks are found.
- 9.5.4.7. Adjust retentate valve restriction by slowly turning retentate valve clockwise until the retentate pressure gauge reads 0.69 Bar (10 psi).
- 9.5.4.8. Adjust pump speed and retentate valve restriction to achieve 2.07 Bar (30 psi) feed pressure and 0.69 Bar (10 psi) retentate pressure.
- 9.5.4.9. Allow to run until 50 mL remains in the chamber.
- 9.5.4.10. Disconnect the pump outlet (Sta-pure, white) tubing from the pump outlet port and place in waste collection vessel.
- 9.5.4.11. Disconnect the retentate silicone (translucent) tubing from the RET IN port. Fluid should now drain by gravity. If additional drainage is required, a syringe can be placed on the end of the retentate tube and fluid can be blown down.
- 9.5.4.12. Remove the remainder of water in the chamber as follows: Replace retentate tubing (silicone, translucent) in retentate port. Reconnect pump outlet tubing (Sta-Pure, white).
- 9.5.4.13. Disconnect FEED IN tubing and place in collection vessel. Open tank outlet valve, turn pump speed up to drain reservoir.
- 9.5.4.14. Reconnect the pump outlet tubing (Sta-Pure, white) to the Feed In port.
- 9.6. Pre-conditioning
 - 9.6.1. Place end of permeate tubing silicone (translucent) in the waste collection vessel.
 - 9.6.2. Remove reservoir cover and fill the reservoir with 50 mL of PBS containing 0.1% Tween 80 (or other appropriate buffer) and then remove the Vent port plug.
 - 9.6.3. Open the tank outlet valve. Turn the pump on and increase the pump speed until the feed pressure gauge reads 1.38 Bar (20 psi at its maximum; the needle will pulse as the pump turns). Check all system connections for leaks and tighten any connections as necessary.
 - 9.6.4. Continue pumping to the waste collection vessel until the level in the reservoir drops to the bottom of the reservoir stir bar well making sure to stop the pump before air is pumped into the system. Turn the pump off.
- 9.7. Transfer of culture from bioreactor to centrifuge bottles.
 - 9.7.1. Refer to the SOP: Applikon Bioreactor Controller Operation for instructions on removing the headplate of the bioreactor, providing access to the cells and conditioned medium.
 - 9.7.2. Transfer the culture to three 250 mL centrifuge bottles using a 50 mL pipet and PipetAid. Residual culture can be transferred to an Ehrlenmeyer flask for temporary storage.

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- 9.7.3. Centrifuge cells in pre-chilled Sorvall centrifuge, fitted with a SLA1500 rotor, at 500 x g for 5 min, 4 degrees C.
 - 9.7.4. Transfer conditioned medium (CM) from centrifuge bottle to storage vessel/bottle by carefully decanting the supernatant to appropriately labeled 250 mL Corning bottles.
 - 9.7.5. Add protease inhibitors and Tween 80 as follows. To each 250 mL bottle of CM supernatant, add 1 mL 10mg/mL PMSF, 50 µl of 10 mg/mL Aprotinin stock and 62.5 µl 2 mg/mL Leupeptin stock. Also add 2.5 mL 10% Tween 80 (final concentration will be 0.1%).
 - 9.7.6. Sterile filter the conditioned media using the vacuum sterile filter unit with storage bottle in the BSC. Store the Conditioned Media at 4°C for further processing.
- 9.8. Concentrate the Sample
- 9.8.1. Remove the reservoir cover and fill the reservoir with the sterile filtered conditioned media (up to 500 mL) to be concentrated.
 - 9.8.2. Ensure the vent port is open by removing the plug from the VENT port and leaving it open or installing a Millex Filter if required. Open the tank outlet valve.
 - 9.8.3. Turn the pump on and increase the pump speed until the feed pressure gauge reads 1.38 Bar (20 psi). Check all system connections for leaks and tighten any connections as necessary.
 - 9.8.4. Adjust the retentate valve restriction by slowly turning the retentate valve clockwise until the retentate pressure gauge reads 0.69 Bar (10 psi).
 - 9.8.5. Adjust the pump speed and retentate valve restriction to achieve desired feed retentate pressures [2.07 Bar (30 psi feed / 0.69 Bar (10 psi) retentate)]. Do not exceed 4.14 Bar (60 psi) feed pressure.
 - 9.8.6. Concentrate the solution until the desired volume is reduced 10 fold or greater, but ideally down to about 20 mL.
 - 9.8.7. Turn off the pump and empty the permeate container into a large bottle with a cap and label as:Permeate Waste; bleach then dispose off.
- 9.9. Retrieve the Sample
- 9.9.1. Disconnect the pump outlet tubing (Sta-Pure, white) from pump outlet port and place in product recovery collection vessel (50 mL conical tube).
 - 9.9.2. Disconnect the retentate tubing (silicone, translucent) from the retentate in port and open back pressure regulation valve (turn counterclockwise). Fluid should now drain by gravity.
 - 9.9.3. When drainage ceases, rinse the Pellicon innards by injection of 5 mL of 1X PBS with 0.1% Tween 80 from the retentate tube using a 10 mL syringe. If additional drainage is required; a syringe can be placed on the end of the retentate tube and fluid can be blown down.
 - 9.9.4. Replace retentate tubing (silicone, translucent) in retentate port. Reconnect pump outlet tubing (Sta-Pure, white).
 - 9.9.5. Disconnect FEED IN tubing and place in collection vessel. Open tank outlet valve, turn pump speed up to drain reservoir.

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- 9.9.6. Stop the pump.Reconnect the pump outlet tubing (Sta-Pure, white) to the Feed In port.Close the tank outlet valve.
- 9.9.7. Add 10 ml 1X PBS/0.1% Tween 80 to the reservoir. Open the tank outlet valve.
- 9.9.8. Connect the male luer end of the permeate tubing to the recirculation (DIA / RECIRC) port. Turn the pump on and increase the pump speed until the feed pressure gauge reads 1.38 Bar (20 psi). Check all system connections for leaks and tighten any connections as necessary.
- 9.9.9. Adjust the retentate valve restriction by slowly turning the retentate valve clockwise until the retentate pressure gauge reads 0.69 Bar (10 psi). Adjust the pump speed and retentate valve restriction to achieve 2.07 Bar (30 psi) feed pressure and 0.69 Bar (10 psi) retentate pressure.
- 9.9.10. Recirculate the solution for 10 minutes and then turn the pump off
- 9.9.11. Disconnect the male luer end of permeate tubing from thr recirculation port.
- 9.9.12. Disconnect the pump outlet tubing (Sta-Pure, white) from pump outlet port and place in product recovery collection vessel (50 mL conical tube).
- 9.9.13. Disconnect the retentate tubing (silicone, translucent) from the retentate in port and open back pressure regulation valve (turn counterclockwise). Fluid should now drain by gravity.
- 9.9.14. When drainage ceases, if additional drainage is required; a syringe can be placed on the end of the retentate tube and fluid can be blown down.
- 9.9.15. Replace retentate tubing (silicone, translucent) in retentate port. Reconnect pump outlet tubing (Sta-Pure, white).
- 9.9.16. Disconnect FEED IN tubing and place in collection vessel. Open tank outlet valve, turn pump speed up to drain reservoir.Stop the pump
- 9.9.17. Reconnect the pump outlet tubing (Sta-Pure, white) to the Feed In port.Close the tank outlet valve.
- 9.9.18. Stop the pump, close the outlet valve, and add 5 mL 1X PBS/Tween80 to the chamber. Pipet the solution along the walls repeatedly to rinse, then collect and transfer to the collection vessel.
- 9.9.19. Label the recovery collection vessel Concentrated ant-IL8, [date], [initials] [Volume of mAb retrieved].
- 9.10. Flushing
 - 9.10.1. To begin cleaning the Millipore TFF apparatus and Pellicon filter, repeat Flushing as described in 9.8.4
- 9.11. Cleaning the Labscale TFF/Pellicon cassette.
 - 9.11.1. Disconnect the retentate tubing (silicone, translucent) from RET IN port and place in waste collection vessel. Place the end of the permeate tubing in the waste collection vessel.
 - 9.11.2. Open the retentate valve by turning it counterclockwise.
 - 9.11.3. Remove the reservoir cover and fill with 500 mL of 0.1N NaOH. Ensure the vent port is open by removing the plug from the VENT port and either leave open or install a Millex Filter.
 - 9.11.4. Open the tank outlet valve.

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- 9.11.5. Turn the pump on and increase the pump speed until the feed pressure gauge reads 1.38 Bar (20 psi). Check all system connections for leaks and tighten any connections as necessary.
- 9.11.6. Continue pumping to the waste collection vessel until the level in the reservoir drops to 250 mL and then turn the pump off. Reconnect the retentate (silicone, translucent) tubing to the RET IN port.
- 9.11.7. Recirculate the cleaning solution for 30-60 minutes and then turn the pump off.
- 9.12. Drain the System
 - 9.12.1. Disconnect the pump outlet (Sta-pure, white) tubing from the pump outlet port and place in waste collection vessel.
 - 9.12.2. Disconnect the retentate silicone (translucent) tubing from the RET IN port. Fluid should now drain by gravity. If additional drainage is required, a syringe can be placed on the end of the retentate tube and fluid can be blown down.
- 9.13. Flushing
 - 9.13.1. Repeat Flushing as described in 9.4.4.
- 9.14. Pellicon XL Device Storage
 - 9.14.1. Turn all of the lock nuts until you are able to remove the Pellicon XL Device.
 - 9.14.2. Fill a 10 mL syringe with 0.05N NaOH Storage solution.
 - 9.14.3. Place the cassette in sink or tray that can contain any overflow. Attach the syringe to the retentate port and slowly push the solution into the device. Remove the syringe and replace all of the plugs on the ports and store flat at 4°C-25°C.
- 9.15. Clean Base
 - 9.15.1. Disconnect the power cord.
 - 9.15.2. Clean exterior surfaces, reservoir, and Labscale System Base with a mild soap and water solution.

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10. Attachments:

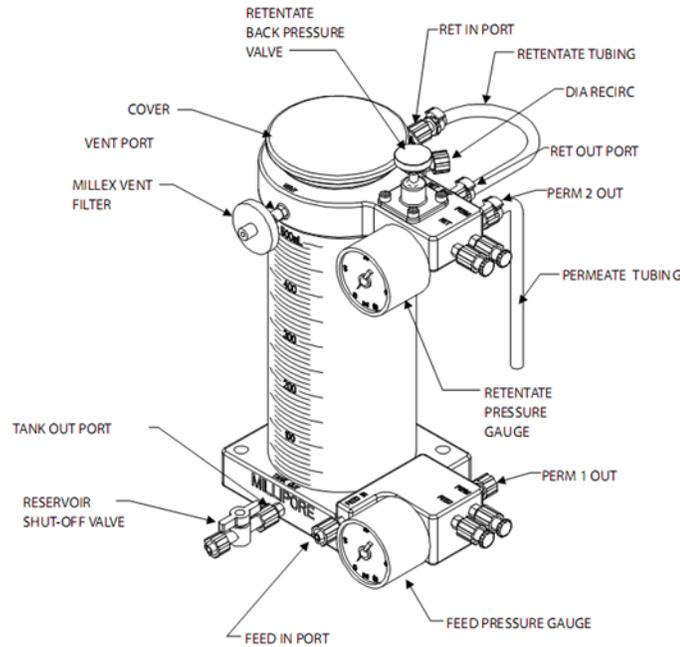


Figure 1: Reservoir Set Up
(<http://www.millipore.com/userguides.nsf/docs/p60085>)

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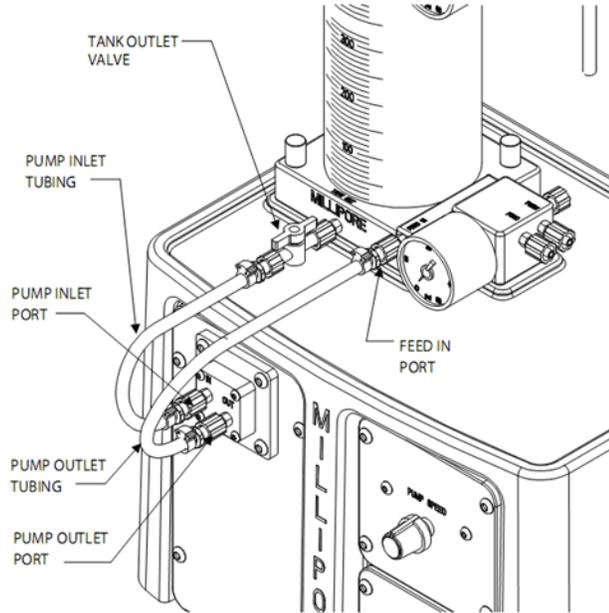


Figure 2: Pump Base Set Up
<http://www.millipore.com/userguides.nsf/docs/p60085>

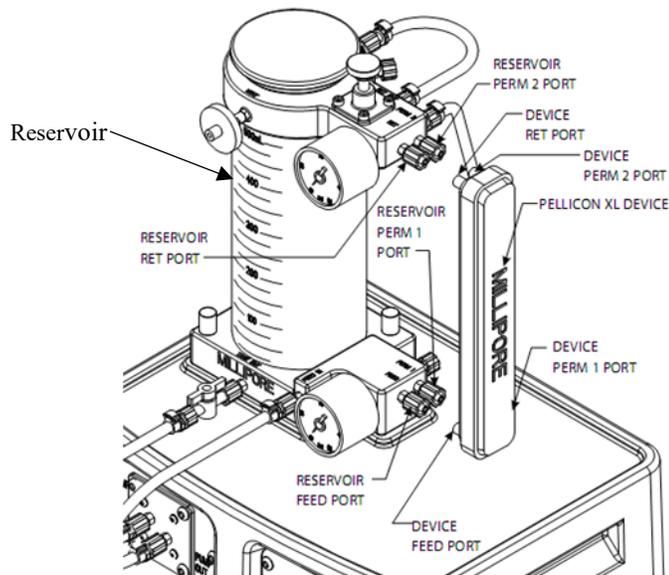


Figure 3: Installation of Pellicon XL Device
<http://www.millipore.com/userguides.nsf/docs/p60085>

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History

<i>Revision Number</i>	<i>Effective Date</i>	<i>Preparer</i>	<i>Description of Change</i>
0	16JUN17	Hetal Doshi	Initial release
1	20DEC18	Hetal Doshi	Added buffer exchange step.
2	25MAR20	Hetal Doshi	Removed buffer exchange step Added recirculation of 1X PBS with 0.1% tween 80 to retrieve the bound mAb from pelican cassette step

SOP: Purification of Anti IL-8 from Conditioned Medium by Protein A Chromatography on the ÄKTA PURE System

Approvals

Preparer: Dr. David Frank
Reviewer: Jason McMillan
Reviewer: Hetal Doshi
Reviewer: Dr. Maggie Bryans

Date: 20APR16
Date: 21APR16
Date: 08JAN20
Date: 10JAN20

1. Purpose

1.1. This procedure describes the isolation of monoclonal antibody from conditioned medium (produced by CHO cells expressing recombinant anti-IL-8) using Protein A affinity chromatography with the ÄKTA pure Chromatography System, controlled by Unicorn 6.3 software.

2. Scope and Applicability

2.1. Applies to purification of mAb from prepared conditioned medium, which has been concentrated and its buffer exchanged by ultrafiltration. The method employs a 1 ml HiTrap Protein A-HP column installed on the GE ÄKTA pure Chromatography System and controlled by Unicorn 6.3 software.

3. Summary of Method

- 3.1. Preparation of buffer(s)
- 3.2. Equilibration of system and column
- 3.3. Fraction collector setup
- 3.4. Application of sample to affinity column
- 3.5. Washing and elution of column
- 3.6. Regeneration of system in preparation for subsequent run
- 3.7. Procedures for short or long-term storage of the system

4. References

- 4.1. Unicorn 6.3 Users Guide (electronic)
- 4.2. AKTA pure 25 Users Guide (electronic)
- 4.3. HiTrap Protein A HP 1 ml column information booklet (GE Healthcare)

5. Definitions

- 5.1. N/A

6. Precautions

- 6.1. Routine care should be exercised in handling of buffers and samples of biological materials, which may have harmful biological activity in the case of accidental ingestion, needle stick, etc.
- 6.2. User should read and be familiar with general good practice as outlined in the AKTA pure Cue Cards located near the instrument.
- 6.3. Avoid damaging the threads through the use of excessive force when connecting plastic fasteners.
- 6.4. It is imperative that pumps never be allowed to run dry. Care must be taken to avoid air in the fluid path, which could damage the pumps or give spurious and uninterpretable readout from the UV and/or conductivity detectors. In order to prevent this prime the

SOP: Purification of Anti IL-8 from Conditioned Medium by Protein A Chromatography on the ÄKTA PURE System

pump rinsing system, prime inlets and purge pump heads before each run by referring to the steps 9.5 and 9.6 of the SOP: Operation of ÄKTA pure Chromatography System.

- 6.5. Gloves and protective eyewear should be worn when handling samples and reagents (buffers), however it is preferable that the user remove gloves prior to entering commands via the computer keyboard or mouse.
- 6.6. Buffers must be degassed and filtered prior to use with the AKTA pure instrument. Samples should be, at a minimum, centrifuged at 10000xg for 5 min before injection/introduction into the fluid path.
- 6.7. Equipment calibration check: The AKTA pure system calibration is automatic; baseline for measurements of A280 and conductivity are zeroed at the beginning of a chromatography run. Calibration of the pH detector is described below.

7. Responsibilities

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

8. Equipment and Materials

- 8.1. AKTA pure chromatography system
- 8.2. HiTrap Protein A HP (1 ml) column; stored at 4°C- bring to room temperature prior to installation (get it out now).
- 8.3. Additional Lab Equipment: pH meter, balance, table top centrifuge with swinging bucket rotor
- 8.4. Lab Utensils: Beakers (250, 500ml, 1200 ml), 1 liter and 500 ml graduated cylinders
- 8.5. Reagents:
 - 8.5.1. 20 mM sodium phosphate buffer, pH 7.0
 - 8.5.2. 0.1 M sodium citrate, pH 3.0
 - 8.5.3. 1 M Tris base pH9.0
 - 8.5.4. Filtered deionized water (MilliQ or similar).
 - 8.5.5. 20% Ethanol
 - 8.5.6. 10N NaOH
 - 8.5.7. pH Standard buffers: pH 7, pH 4.01
 - 8.5.8. Stock solutions of protease inhibitors: 10 mg/ml PMSF in isopropanol, 2 mg/ml leupeptin, 10 mg/ml aprotinin.
- 8.6. Lab Supplies:
 - 8.6.1. Filters (0.2 µm); (3 bottle top; 2 syringe mounted)
 - 8.6.2. Corning bottles for vacuum filtration, degassing of all chromatography buffers.
 - 8.6.3. Syringe with leur lock (10 ml) – (2).
 - 8.6.4. Tubes for fraction collector – (30)
 - 8.6.5. Graduated cylinders: 1L, 250 ml, 100 ml
 - 8.6.6. Beakers: 1L, 400 ml, 200 ml

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9. Procedure

9.1. Reagent Preparation:

9.1.1. Buffer A: Binding buffer: 20 mM sodium phosphate, pH 7.0

9.1.1.1. Weigh 1.084 ± 0.02 g NaH_2PO_4 and transfer to a 1200 ml beaker with magnetic stir bar.

9.1.1.2. Weigh 3.2 ± 0.02 g $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ and transfer to the same beaker.

9.1.1.3. Measure 980 ml MilliQ water in a graduated cylinder and add the water to the solids in the flask.

9.1.1.4. Stir until the solids have dissolved, check the pH and adjust the pH to 7.0 if required with 1N phosphoric acid.

9.1.1.5. Transfer to a 1L graduated cylinder and adjust the final volume to 1L.

9.1.1.6. Sterile filter the solution, allowing it to degas for 15-20 minutes. Label appropriately.

9.1.2. Buffer B: Elution buffer: 0.1M sodium citrate, pH 3.0

9.1.2.1. Weigh 3.84g citric acid in a 400 ml beaker with magnetic stir bar.

9.1.2.2. Dissolve in 180 ml MilliQ water.

9.1.2.3. Adjust the pH dropwise with 10N NaOH, to a final pH of 3.0

9.1.2.4. Transfer the solution to a 250 ml graduated cylinder. Adjust the final volume to 200 ml.

9.1.2.5. Filter the solution, allowing it to degas for 15 – 20 minutes.

9.1.2.6. Label appropriately.

9.1.3. 1M Tris base pH9.00: added to fraction collector tubes to rapidly neutralize acid-eluted fractions from the protein A column.

9.1.3.1. Weigh 12.11 gm Tris base [tris(hydroxymethyl)aminomethane] into a plastic weigh boat and transfer to a 200 ml beaker with a stir bar.

9.1.3.2. Measure 90 ml MilliQ water in a graduated cylinder and transfer the water to the beaker containing Tris powder. Stir until dissolved, then adjust pH to 9.0

9.1.3.3. Transfer the Tris solution quantitatively to a 100 ml graduated cylinder, rinsing the beaker with small aliquots of water, which are then added to the cylinder until a final volume of 100 ml is obtained.

9.1.3.4. Filter the solution. Degassing is not necessary.

9.2. Calibration of the pH Electrode

Calibration of the pH detector is performed daily, when the instrument is in use. The calibration procedure utilizes ordinary pH standards found in the lab. Calibration is dictated by a method built into Unicorn.

9.2.1. Obtain three small beakers and pH standards for pH 4.01 and pH 7.0, as well as a 10 ml syringe and a bottle of MilliQ water.

9.2.2. In the Unicorn System Control window, choose 'Calibration' from the System menu. From the drop-down menu under 'Monitor to calibrate', select 'pH'.

9.2.3. Click the 'Prepare for Calibration' button. You will hear the valve switch to the calibrate position.

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- 9.2.4. Follow the on-screen instructions for both pH standards. Enter the pH of the first pH standard buffer in the *pH for buffer 1* field.
- 9.2.5. Fill a syringe with approximately 10 ml of the first pH standard buffer (pH 7). Connect the syringe to the Luer connector of pH valve port **Cal**, and inject the buffer. When the *Current value* is stable, click the *Calibrate* button.
- 9.2.6. Thoroughly rinse the syringe with 3-4 changes of MilliQ water. Wash the pH flow cell by injecting water into pH valve port **Cal**.
- 9.2.7. Enter the pH of the second pH standard buffer in the *pH for buffer 2* field. Fill a syringe with approximately 10 ml of the second pH standard buffer. Connect the syringe to the Luer connector of pH valve port **Cal**, and inject the buffer. When the *Current value* is stable, click the *Calibrate* button.
- 9.2.8. The calibration date and time are displayed in the dialog, along with values for *Calibrated electrode slope* (should be $\geq 80\%$) and *Asymmetry potential at pH 7* (should be within the interval ± 60 mV. If the conditions are met, click the *Close* button to switch the pH valve back to the default position and to close the *Calibration* dialog.
- 9.2.9. If values for the slope and potential are not within acceptable values, clean the pH electrode and repeat the calibration procedure. If this does not help, replace the electrode.
- 9.3. Start-up and preparation of AKTA pure Instrument and computer:
 - Degassed buffers should be in place prior to turning on the AKTA pure instrument. Equipment start-up requires turning on the instrument and, separately, the computer connected to it.
 - 9.3.1. Place the degassed buffers A and B on top of the AKTA pure instrument.
 - 9.3.2. Locate Inlet tubing A1 and B1 (atop the instrument and resting in water or 20% ethanol). Each has a filter unit attached, which distinguishes them from A2 and B2; those end in a male threaded fitting and will not be used for this procedure.
 - 9.3.3. Transfer tubing Inlet A1 to the buffer A bottle.
 - 9.3.4. Transfer tubing Inlet B1 to the buffer B bottle.
 - 9.3.5. The On/Off switch for the instrument is located on the right side toward the rear of the housing. Switch to the 'On' position. Audible emanations from within the instrument cabinet indicate that the AKTA pure system is going through its brief initialization sequence.
 - 9.3.6. The computer On/Off switch is located on the front of the Dell desktop computer unit, near the top of the case. Press in to turn on the computer.
 - 9.3.7. Login to the computer using credentials provided by the College.
 - 9.3.8. Double click the Unicorn 6.3 icon on the desktop to open the software which controls the instrument functions. Click OK in the "Log In – Unicorn" dialog box that appears.
 - 9.3.9. Open the System Control window (under Tools menu, if not opened automatically on startup).
 - 9.3.10. The top pane of the window will show the current state of the instrument, and the bottom pane shows the fluid path and manual controls. If the window is blank, go to

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the System menu and select Connect to Systems, check the box by AKTA pure 25 and click OK.

- 9.3.11. Confirm that the correct column (HiTrap Protein A-HP 1 ml) is attached to the system. If not, refer to Section 9.4 (Installing/Changing a Chromatography Column on the AKTA pure Chromatography System).
- 9.3.12. Under the File menu, choose Open and select the method with file name “*HiTrap Protein A 1ml Equilibration*”.
- 9.3.13. A dialog box appears that allows the method to be run. Click Start to initiate flushing of the pumps and equilibration of the column.
- 9.3.14. While the equilibration method is running, prepare the fraction collector for later steps by filling the carousel with clean tubes. Add 200µl 1M Tris to the bottom of each tube – this serves to rapidly neutralize the acidic eluent (which destabilizes some antibodies).
- 9.3.15. Allow the program to run to completion, about 15 minutes.
- 9.4. Installing/Changing a Chromatography Column on the AKT Apure Chromatography System.

It is imperative that the following operations be performed in such a way as to prevent the introduction of air bubbles into the column, which is achieved by making liquid-to-liquid (drop-to-drop) contact prior to inserting the threaded fitting into its position.

 - 9.4.1. Confirm that the Protein A column has reached room temperature.
 - 9.4.2. Have on hand a few paper lab towels and a 250ml beaker to catch waste.
 - 9.4.3. Remove tube connector from the UV detector inlet by unscrewing the knurled fastener.
 - 9.4.4. Initiate flow manually at 0.5 ml/min collecting waste in the beaker or towel.
 - 9.4.5. Remove the plug from the column inlet and place a few drops of 20% ethanol in the inlet, filling it to insure the absence of air.
 - 9.4.6. As a droplet emerges from the inlet tubing, touch it to the liquid in the column inlet and begin to thread the fitting in, leaving slight looseness of threads so that liquid escapes around the fitting and pressure buildup in the column is prevented.
 - 9.4.7. Remove the column bottom plug and screw the column directly into the UV detector inlet.
 - 9.4.8. Tighten the column inlet fitting just enough to prevent leaking.
 - 9.4.9. The column is now ready to equilibrate in buffer (step 9.3.12) prior to performing a chromatography run.
- 9.5. Performing a chromatography run:
 - 9.5.1. Place the fraction collector tube 1 near the outlet tubing from the instrument (refer to attachment Fig 1) so that it will touch the arrow on the white paddle of the fraction collector arm. Note: To rotate the carousel, reach around the left side of the collector to find a rubber roller pressing against the carousel (Fig 2). Pull the roller away from the carousel (Fig. 3); the carousel will rotate freely as long as the roller is held. When the first tube is in the correct position, release the roller.
 - 9.5.2. Gently raise the arm and swing it into position against tube 1.

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- 9.5.3. Place all 'Waste' tubing, labeled W, W1 & W2 in 500ml Erlenmeyer flask.
- 9.5.4. Place the tube labeled Outlet in a 125ml Erlenmeyer flask.
- 9.5.5. Fill a 10 ml syringe with the mAb sample (concentrated, filtered conditioned medium), expel any bubbles and insert the loaded syringe into the injection port.
- 9.5.6. Inject the sample into the port to fill the 10ml Superloop.
- 9.5.7. Open the Unicorn software and navigate to the System Control window.
- 9.5.8. Under the File menu, choose Open and select the method with file name "*1ml Protein A Column ver2*".
- 9.5.9. In the dialog box that opens, enter operator's name, sample notes.
- 9.5.10. Click Next; take note of the time and volume for the run; make sure there is excess buffer A and B.
- 9.5.11. Click Next. Enter the buffer composition of each buffer and the sample identity.
- 9.5.12. Click Next. Enter a filename composed of the method name, date, operator or group initials, for example **HiTrapProtA antiIL8 16May15 SDBiopharm**.
- 9.5.13. Click Start. The instrument should begin to execute the method, as evidenced by a soothing hum from the pumps and drops of liquid falling into tube 1 from the fraction collector outlet.
- 9.5.14. Observe that the fraction collector is receiving drops.
- 9.5.15. Monitor the computer screen for error messages or warnings.
- 9.5.16. Allow the method to run to completion, at which time the system will be re-equilibrated and ready for subsequent runs by repeating section 9.6
- 9.5.17. Remove tubes from the fraction collector and place in a rack for storage at 4°C, awaiting further analysis. Cover the top of the tubes with lab film. The peak of absorbance at 280 nm which eluted with the low pH buffer B contains purified mAb, which will be examined by the QC Biochemistry Dept.
- 9.6. Equipment shut-down and short term (less than 3 days) storage
 - 9.6.1. After completion of the final separation of the day, transfer Inlet tubing A1 and B1 to a flask of degassed Milli-Q water (250ml or greater).
 - 9.6.2. In the Unicorn software, open the System Control window.
 - 9.6.3. Under the File menu, choose Open, then select the method 'System Short Term Storage'.
 - 9.6.4. Click Start.
 - 9.6.5. Allow the method to run to completion, as indicated by an audible tone and onscreen window.
 - 9.6.6. Turn off the instrument or perform the long-term storage routine as needed (section 9.6).
- 9.7. Cleaning of the Superloop 10 sample holder.
 - 9.7.1. For short term storage of the Superloop on the AKTA instrument, inject 2 ml Milli-Q water into the sample chamber.
 - 9.7.2. Pump it out to waste by temporarily disconnecting the outlet tubing that is connected to the injection valve at port 'loop F'.

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- 9.7.3. Using manual control in the System Control window of Unicorn, set the flow rate to 2 ml/min and the injection valve position to Inject. Allow pump A to run until the Superloop chamber is empty.
- 9.7.4. Reconnect to 'loop F'.
- 9.7.5. Repeat steps 9.6.1 thru 9.6.4 three times.
- 9.7.6. Inject 10 ml water into the sample chamber of the Superloop.
- 9.8. Equipment shut-down and long term (3 days or more) storage
 - 9.8.1. After completion of the System Short Term Storage method, transfer Inlet tubing A1 and B1 to a flask of degassed 20% ethanol (250ml or greater).
 - 9.8.2. In the Unicorn software, open the System Control window.
 - 9.8.3. Confirm that the pH valve is in the 'Bypass' or 'Restrictor' position.
 - 9.8.4. Under the File menu, choose Open, then select the method 'System Long Term Storage'.
 - 9.8.5. Click Start.
 - 9.8.6. Allow the method to run to completion, as indicated by an audible tone and onscreen window.
 - 9.8.7. Referring to section 9.4 for guidance, remove the HiTrap Protein A-HP column from the instrument and cap both ends, taking care to avoid introduction of air into the column. Store the column in a refrigerator.
 - 9.8.8. Turn off the AKTA pure instrument.
- 9.9. Printing Your Chromatogram
 - 9.9.1. In the Unicorn software interface, open the Evaluation window.
 - 9.9.2. In the Result Navigator pane, click the Results tab.
 - 9.9.3. Locate the file of interest and double click its name to display your chromatogram in the right pane.
 - 9.9.4. *Optional:* Click the Customize button to open a dialog box that allows you to specify what curves display and the scale of each axis. Recommended are the UV Chrom curve, Conductivity, and Fraction Number.
 - 9.9.5. Click the Report button, check the Default report in the selection window and click Preview.
 - 9.9.6. Under File, choose to Print (or Save as PDF to use a different printer).

SOP: Purification of Anti IL-8 from Conditioned Medium by Protein A Chromatography on the ÄKTA PURE System

Attachments/Figures

Fig. 1. Diagram of AKTA pure instrument, fraction collector and computer

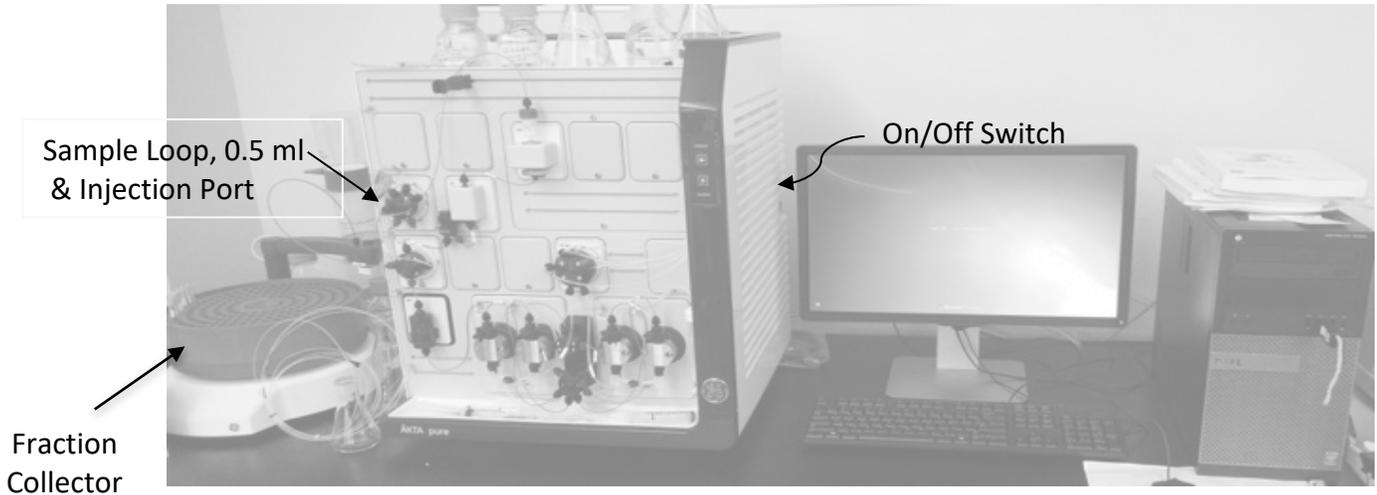
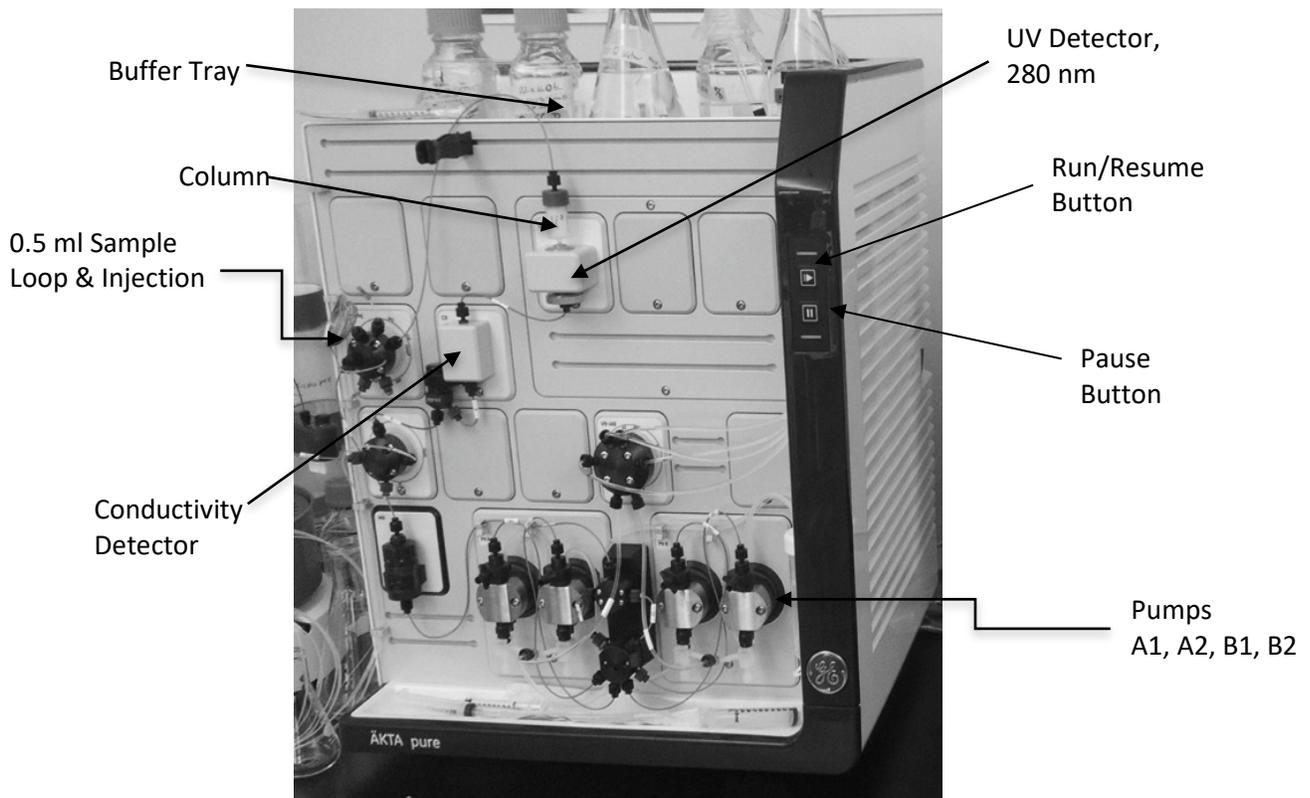


Fig. 2. AKTA pure Instrument Features



SOP: Purification of Anti IL-8 from Conditioned Medium by Protein A Chromatography on the ÄKTA PURE System

Fig 3. *System Control window within the Unicorn 6.3 software.*

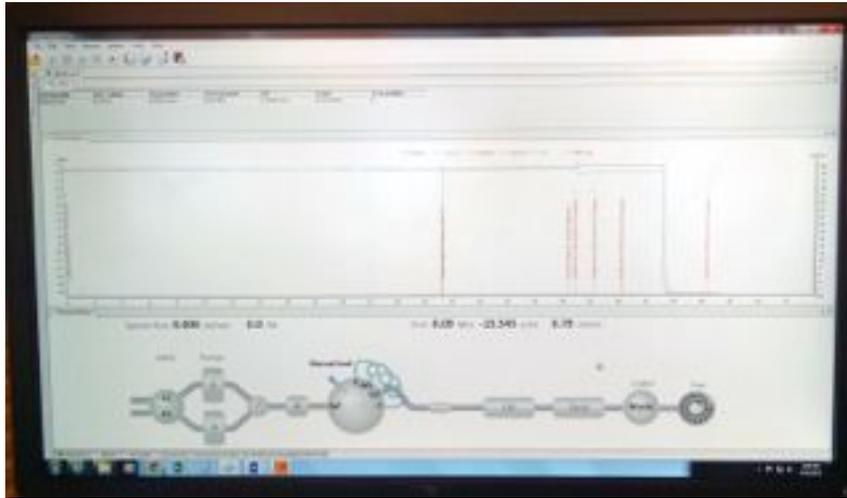
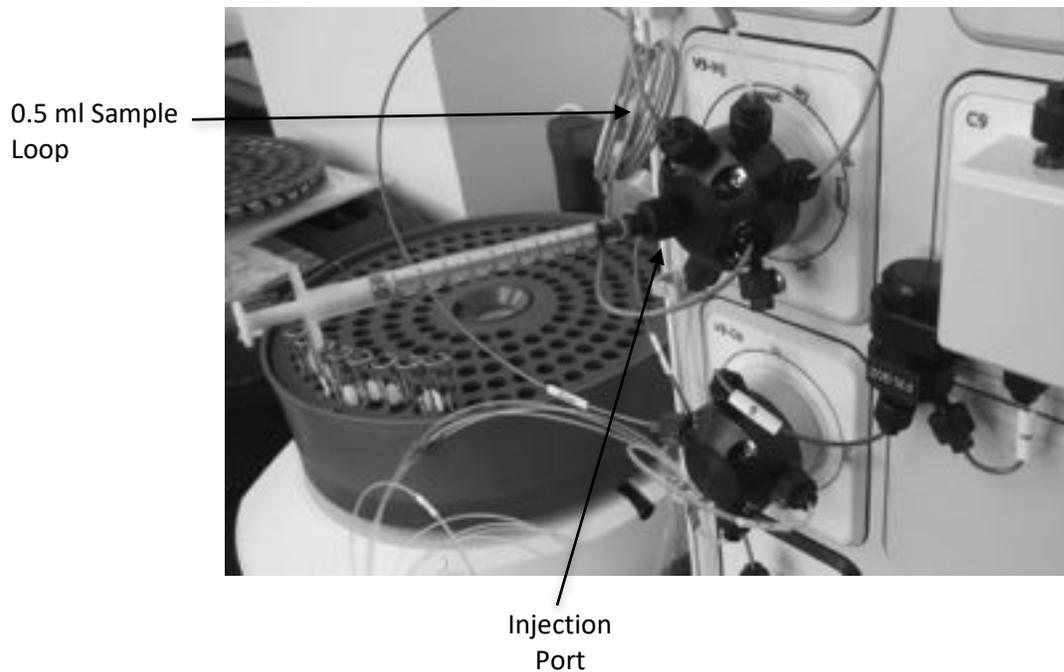


Fig. 4. *Detail of Injection Port with Syringe in Place.*



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Fig. 5. *Fraction collector carousel rubber advancement roller/gear.*

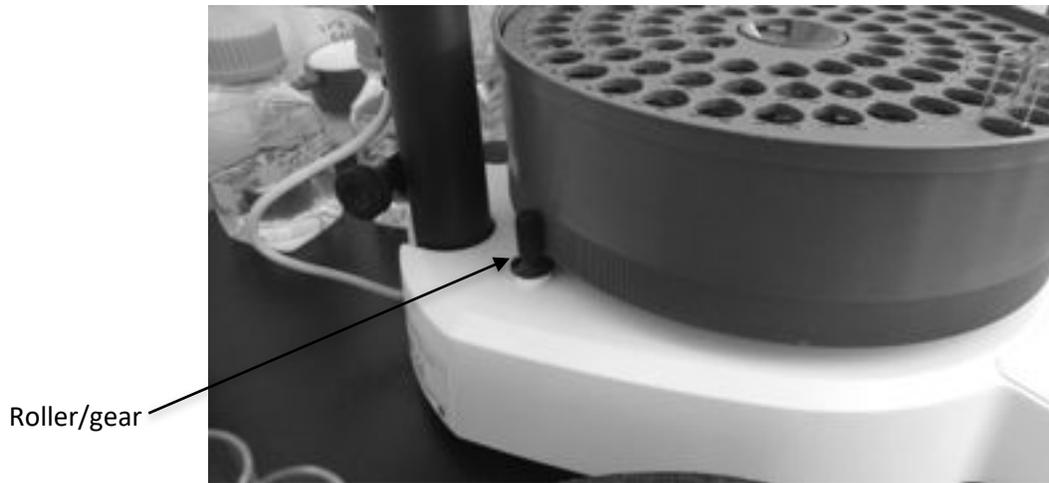
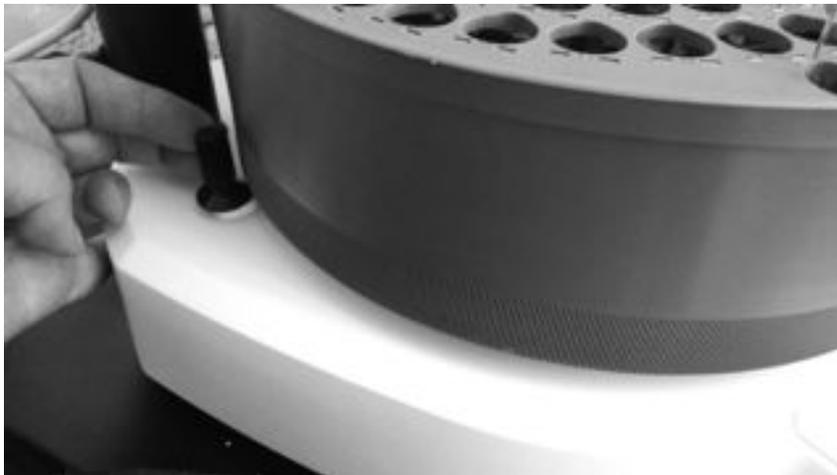


Fig. 6. *Release of roller to allow free rotation of the carousel.*



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Fig. 7. Location of tube #1 under the fraction collector drip outlet.



10. History

<i>Revision Number</i>	<i>Effective Date</i>	<i>Preparer</i>	<i>Description of Change</i>
0	21APR16	David Frank	Initial release
1	19FEB18	Hetal Doshi	Removed concentrating of the cell suspension step and Changed the neutralizing buffer pH to 9.0
2	10JAN20	Hetal Doshi	Added the note about priming the pump rinse system, priming the inlets and purging the pump heads to the precaution section Change the method to 1ml protein A column ver2

Batch Record for Downstream Processing of Anti IL-8 mAb

Approvals:

Preparer: Jason McMillan & Dr. David Frank
 Reviewer: Hetal Doshi
 Reviewer: Dr. Margaret Bryans

Date: 15APR16
 Date: 08JAN20
 Date: 10JAN20

1.0 Harvest, Centrifugation, Concentration and Buffer exchange

1.1 Description

This batch record directs and documents the isolation of Anti IL-8 mAB from conditioned medium of producer CHO cells grown in a bioreactor, providing bench scale Downstream Processing procedures to :

- 1). Clarify conditioned medium by centrifugation to remove cells and debris
- 2). Concentrate and perform buffer exchange of anti IL-8 mAB in conditioned growth medium by tangential flow filtration

The method demonstrates the principles of Centrifugation and Tangential Flow Filtration.

1.2 References

<i>Title</i>	<i>Doc #</i>
Millipore Tangential Flow and Diafiltration Using Pellicon XL Device of tPA SOP	DP 1
SOP: End-of-Run Anti IL-8 mAB Process: Harvest, Centrifugation, TFF concentration	DP
URL for Labscale User Guide and Documentation: http://www.emdmillipore.com/Web-US-Site/en_CA/-/USD/ViewParametricSearch-SimpleOfferSearch?SearchTerm+=labscale++pellicon&SelectedSearchResult=SFdocumentSearch&SearchContextPageletUUID=	N/A

1.3 Equipment

<i>Equipment Type</i>	<i>Manufacturer, Model</i>	<i>ID #</i>	<i>Initials/Date</i>	<i>Verifier/Date</i>
Tangential Flow Filtration System	Millipore Labscale 500ML			
Ultrafiltration Cassette	Millipore Pellicon XL PXC030C50			
Centrifuge	Dupont Sorvall RC5			
Centrifuge Rotor	Sorvall SLA 1500			
Centrifuge Rotor	Sorvall SS-34			

Batch Record for Downstream Processing of Anti IL-8 mAb

1.4 Materials

<i>Component</i>	<i>Quantity Required</i>	<i>Quantity Used</i>	<i>Initials/Date</i>	<i>Verifier/Date</i>
250 ml Nalgene centrifuge bottles	3-4			
Bottle-top vacuum 0.22 µm filtration device	1			
250 ml Corning bottles	3-4			
10ml graduated cylinder	1			
25 ml beaker	1			
50 ml beaker	1			
Nalgene Oak Ridge centrifuge tubes	2-4			

1.5 Solutions

<i>Solution</i>	<i>Volume</i>	<i>Date Prepared</i>	<i>Initials/Date</i>	<i>Verifier/Date</i>
0.1N NaOH (sodium hydroxide)				
0.05N NaOH (sodium hydroxide)				
<u>TFF Buffer A: Binding buffer for Buffer exchange:</u> 20 mM sodium phosphate, pH 7.0 with 0.1% Tween 80				
10% (w/v) Tween 80				
Phosphate buffer containing 0.1% Tween 80 (preconditioning buffer) 50 ml				
Stock solutions of protease inhibitors:				
PMSF (phenylmethylsulfonyl fluoride), 10 mg/ml in isopropanol; 250X				
Leupeptin, 2 mg/ml; 4000x				
Aprotinin, 10 mg/ml, 5000x				
MilliQ Water				

1.6 Procedure:

1.6.1. Preparation of Solutions

	<i>Solution</i>	<i>Initials/Date</i>	<i>Verifier/Date</i>
Step	0.1N NaOH		
1	Weigh approximately 2.5 g NaOH		
2	Transfer the solid NaOH to a 800 ml beaker with stir bar.		

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3	<p>Measure out the volume of Milli-Q water necessary to produce the desire concentration and transfer to the beaker, according to the formula:</p> $Vol, ml = x \div 40 \div 0.1 \times 1000,$ <p>where x = g NaOH measured</p> <p>Record the following:</p> <p>NaOH measured: _____ g</p> <p>Volume: _____ ml</p>		
4	Store in a clean bottle labelled appropriately		

	0.05N NaOH	Initials/Date	Verifier/ Date
1	Pipet 5 ml MilliQ water into a 15 ml plastic conical tube with screw cap.		
2	Pipet 5 ml 0.1N NaOH into the same tube, cap, mix and label appropriately.		
	10% w/v Tween 80	Initials/Date	Verifier/ Date
1	Measure approximately 80 ml MilliQ water and a magnetic stir bar into a 200 ml beaker.		
2	Place the beaker on a balance and tare the balance when stable.		
3	Pour 10 g Tween 80 (polyoxyethylene sorbitan monooleate) into the beaker with water.		
4	Stir until all of the Tween 80 is dissolved; this can take 30 minutes or more to complete. Carefully adjust the stir plate rpm to provide adequate mixing vigor without introducing air bubbles or frothing.		
5	Quantitatively transfer the solution to a 100 ml graduated cylinder, rinsing the beaker walls with a small amount of MilliQ water (which is then added to the cylinder).		
6	Adjust the final volume to 100 ml.		
7	Store the solution in an appropriately labeled bottle at room temperature.		
	1X PBS with 0.1% Tween 80	Initials/date	
1	Measure 79.2 ml of 1X PBS with 50 ml graduated cylinder		

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2	Transfer the 79.2ml of measured 1X PBS into a clean beaker		
3	Measure 0.8ml of 10% v/v Tween 80 with a serological pipete and add to the 1X PBS		
4	Stir until all of the Tween 80 is dissolved with a magnetic stirrer and stirrer plate		
5	Transfer the prepared 1X PBS with 0.1% tween 80 into clean labelled bottle		
6	Store the solution at room temperature		

	<i>Solution</i>	<i>Initials/Date</i>	<i>Verifier/Date</i>
Leupeptin, 2 mg/ml			
1	Obtain a 5 mg vial of leupeptin. Open carefully with gloved hands in a fume hood.		
2	Pipet 2.5 ml MilliQ water into the vial and mix.		
3	Transfer in aliquots of 100 µl to several 1.5 ml tubes.		
4	Store the solution at 4°C for one week or, preferably at -20°C for 6 months.		
Aprotinin, 10 mg/ml			
1	Obtain a vial of 10 mg aprotinin.		
2	Pipet 1 ml MilliQ water into the vial and mix.		
3	Transfer aliquots of 100 µl to 1.5 ml tubes.		
4	Store the solution at 4°C for one week or, preferably at -20°C. Aprotinin is stable at -20°C for at least 6 months.		
PMSF, 10 mg/ml in isopropanol			
<i>PMSF (phenylmethylsulfonylflouride) is toxic and must be handled carefully, with appropriate personal protective equipment, including a dust mask, gloves, lab coat and safety glasses. Open and transfer the powder in the fume hood. The powder may become airborne in the presence of static electricity and should be transferred directly from the bottle to a tube with cap.</i>			
1	Place a 15 ml conical tube with screw cap in a beaker on the pan of a balance capable of weighing mg quantities. Tare the balance.		
2	Working in the fume hood, use a metal spatula to transfer a quantity of PMSF from the bottle into the tube and cap it.		
3	Weigh the tube containing PMSF powder. At least 40 mg will be required for each liter of conditioned medium		
4	Tap the tube to insure the powder is at the bottom of it, then add anhydrous isopropanol, the volume of which is determined by the following equation:		

Batch Record for Downstream Processing of Anti IL-8 mAb

	$Vol, ml = mg \text{ PMSF} \div 10 \text{ mg/ml}$ Record the following: PMSF: _____ mg Isopropanol: _____ ml		
5	Mix to dissolve the powder		
6	Label the tube and store it at 4°C. PMSF is stable in isopropanol (has a very short half life in aqueous solutions).		

1.6.2. Preparation of the Labscale TFF System

<p><i>Note: Become familiar with the location of ports and tubing connection points as shown in the following diagram prior to beginning setup. Also refer to attachment Figures 1-3, found at the end of this document, for correct configuration of the tubing and cassette connections to ports.</i></p>	

#	Task	Initials/ Date	Verifier/ Date
1	If necessary, set up the apparatus and confirm that all tubing connections are secure, according to the SOP (Millipore Tangential Flow and Diafiltration Using Pellicon XL Device of SOP).		
2	Remove the 4 plugs on the Pellicon XL (PXC030C50) cassette ports. Align the Pellicon XL device ports with Labscale 500 ml		

Batch Record for Downstream Processing of Anti IL-8 mAb

	Reservoir ports being sure the PERM and RET DEVICE ports of the Pellicon XL Device and reservoir match. Press the device firmly onto the reservoir ports. Turn the lock nuts until snug.		
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1.6.3. Flushing the Pellicon cassette.

#	<i>Task</i>	<i>Initials/ Date</i>	<i>Verifier/ Date</i>
1	Disconnect retentate silicone (translucent) tubing from RET IN port and place end of retentate tubing in waste collection vessel.		
2	Place end of permeate silicone (translucent) tubing into waste collection vessel. Open retentate valve by turning it counterclockwise.		
3	Remove the reservoir cover and fill reservoir with 500 ml of MilliQ water. Remove the plug from VENT port and open tank outlet valve.		
4	Turn the pump on and increase the speed until the feed pressure gauge reads 20 psi.		
5	Continue pumping to the waste collection vessel until the level in the reservoir drops to 350 ml and then turn the pump off.		
6	Reconnect the retentate silicone (translucent) tubing to the RET IN port and turn the pump on. Slowly increase the pump speed until the feed pressure gauge reads 20 psi. Check the system for leaks and tighten connections if leaks are found.		
7	Adjust the retentate valve restriction by slowly turning the retentate valve clockwise until the retentate pressure gauge reads 10 psi.		
8	Adjust pump speed and retentate valve restriction to achieve 30 psi feed pressure and 10 psi retentate pressure.		
9	Allow to run until 50 ml remains in the chamber, then stop the pump.		
10	Disconnect the pump outlet (Sta-pure, white) tubing from the pump outlet port and place in waste collection vessel.		
11	Disconnect the retentate silicone (translucent) tubing from the RET IN port. Open the retentate backpressure valve by turning counterclockwise. Fluid will now drain by gravity. If additional drainage is required, a syringe can be placed on the end of the retentate tube and fluid can be blown down.		

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12	Remove the remainder of water in the chamber as follows: Replace retentate tubing (silicone, translucent) in retentate port. Reconnect pump outlet tubing (Sta-Pure, white).		
13	Disconnect FEED IN tubing and place in collection vessel. Open tank outlet valve, turn pump speed up to drain reservoir.		
14	Reconnect the pump outlet tubing (Sta-Pure, white) to the Feed In port.		

1.6.4. Pre-conditioning the Pellicon cassette

#	Task	Initials/ Date	Verifier/ Date
1	Place end of permeate tubing silicone (translucent) in the waste collection vessel.		
2	Remove reservoir cover and fill the reservoir with 50 ml of PBS containing 0.1% Tween 80 (or other appropriate buffer) and then remove the Vent port plug.		
3	Open the tank outlet valve. Turn the pump on and increase the pump speed until the feed pressure gauge reads 20 psi at its maximum; the needle will pulse as the pump turns. Check all system connections for leaks and tighten any connections as necessary.		
4	Continue pumping to the waste collection vessel until the level in the reservoir drops to the bottom of the reservoir stir bar well making sure to stop the pump before air is pumped into the system. Turn the pump off. Close the pump outlet valve.		

1.6.5. Clarification of conditioned medium by centrifugation & filtration.

#	Task	Initials/ Date	Verifier/ Date
1	Refer to the SOP: Applikon ez-Control Bioreactor Controller Operation for instructions on removing the headplate of the bioreactor, providing access to the cells and conditioned medium.		
2	Transfer the culture to three 250 ml centrifuge bottles using a 50 ml pipet and PipetAid. Residual culture can be transferred to a clean sterile bottle for temporary storage.		
3	Centrifuge cells in pre-chilled Sorvall centrifuge, fitted with a SLA1500 rotor, at 1000 x g for 5 min, 4 degrees C.		
4	To further clarify the conditioned medium, carefully decant the supernatant into/through a bottle top 0.22µm vacuum filter mounted. Apply the vacuum and complete filtration of the medium.		

Batch Record for Downstream Processing of Anti IL-8 mAb

5	Add protease inhibitors and Tween 80 as follows. To each 250 ml bottle of CM supernatant, add 1 ml 10mg/ml PMSF, 50 µl of 10 mg/ml Aprotinin stock and 62.5 µl 2 mg/ml Leupeptin stock. Also add 2.5 ml 10% Tween 80 (final concentration will be near 0.1%).		
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1.6.6. Concentration of Anti IL-8 mAb in conditioned medium

#	<i>Task</i>	<i>Initials/ Date</i>	<i>Verifier/ Date</i>
1	Make sure the TFF system is flushed and preconditioned		
2	Remove the reservoir cover and fill the reservoir with anti IL-8 mAB sample (up to 500 ml) to be concentrated.		
3	Ensure the vent port is open by removing the plug from the VENT port. Open the tank outlet valve.		
4	Turn the pump on and increase the pump speed until the feed pressure gauge reads 20 psi. Check all system connections for leaks and tighten any connections as necessary.		
5	Adjust the retentate valve restriction by slowly turning the retentate valve clockwise until the retentate pressure gauge reads 10 psi.		
6	Adjust the pump speed and retentate valve restriction to achieve desired feed and retentate pressures: 30 psi feed / 10 psi retentate. Do not exceed 60 psi feed pressure.		
7	Concentrate the solution until the desired volume is reduced 10 fold or greater, but ideally down to about 20 ml.		
8	Turn off the pump and empty the permeate container into a large bottle with a cap and label as: Anti IL-8 Mab, Permeate Waste, disposal; bleach then drain, [initials], [date].		
9	Measure the volume of the retentate in the reservoir with the 25 ml serological pipette		

1.6.7. Recover the concentrated conditioned media

#	<i>Task</i>	<i>Initials/ Date</i>	<i>Verifier/ Date</i>
1	Disconnect the pump outlet tubing (Sta-Pure, white) from pump outlet port and place in product recovery collection vessel (beaker or cleaned sterile 50 ml conical tube).		
2	Disconnect the retentate tubing (silicone, translucent) from the retentate in port and open the retentate back pressure valve (turn counterclockwise). Fluid should now drain by gravity.		

Batch Record for Downstream Processing of Anti IL-8 mAb

3	When drainage ceases, rinse the Pellicon innards by injecting 5 ml of 1X PBS with 0.1% Tween 80 from the retentate tube using a 10 ml syringe. To expel any remaining liquid, use a syringe attached to the end of the retentate tube to force fluid down/out with air.		
4	Replace retentate tubing (silicone, translucent) in retentate port. Reconnect pump outlet tubing (Sta-Pure, white).		
5	Disconnect FEED IN tubing and place in collection vessel. Open tank outlet valve, turn pump speed up and let the reservoir drain.		
6	Stop the pump. Reconnect the pump outlet tubing (Sta-Pure, white) to the Feed In port, Close the tank outlet valve		
7	Add 10 ml of 1X PBS with 0.1% Tween 80 to the reservoir. Open the tank outlet valve		
8	Connect the male luer end of the permeate tubing to the recirculation (DIA / RECIRC) port. Turn the pump on and increase the pump speed until the feed pressure gauge reads 20 psi. Check all system connections for leaks and tighten any connections as necessary.		
9	Adjust the retentate valve restriction by slowly turning the retentate valve clockwise until the retentate pressure gauge reads 10 psi. Adjust the pump speed and retentate valve restriction to achieve 30 psi feed pressure and 10 psi retentate pressure.		
10	Recirculate the cleaning solution for 10 minutes and then turn the pump off.		
11	Disconnect the pump outlet tubing (Sta-Pure, white) from pump outlet port and place in product recovery collection vessel used in step 1 of 1.6.7. (beaker or cleaned sterile 50 ml conical tube). Disconnect the male luer end of the permeate tubing from recirculation port and place it in waste collection vessel.		
12	Disconnect the retentate tubing (silicone, translucent) from the retentate in port and open the retentate back pressure valve (turn counterclockwise). Fluid should now drain by gravity.		
13	When drainage ceases, to expel any remaining liquid, use a syringe attached to the end of the retentate tube to force fluid down/out with air		
14	Replace retentate tubing (silicone, translucent) in retentate port. Reconnect pump outlet tubing (Sta-Pure, white).		
15	Disconnect FEED IN tubing and place in collection vessel. Open tank outlet valve, turn pump speed up and let the reservoir drain.		
16	Stop the pump, close the outlet valve and Reconnect the pump outlet tubing (Sta-Pure, white) to the Feed In port		
17	Label the recovery collection vessel as Concentrated anti_IL8, [date], [initials], company name. Measure and record the volume.		

Batch Record for Downstream Processing of Anti IL-8 mAb

18	Store for the short term (1 week) in 2°C-8°C refrigerator for use in further purification steps. Long term storage is at -20°C.		
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1.6.8. Cleaning the Pellicon XL cassette ultrafiltration membrane.

<p><i>Cleaning of the Pellicon cassette and its internal ultrafiltration membrane is achieved by:</i></p> <ol style="list-style-type: none"> <i>1. flushing the system with MilliQ water (a repeat of procedure 1.6.3)</i> <i>2. cleaning with 0.1N NaOH.</i> <i>3. flushing once more with MilliQ water (procedure 1.6.3).</i> <p><i>Cleaning may be initiated and left to continue while the subsequent operation (chromatography) is performed.</i></p>			
#	Task	Initials/ Date	Verifier/ Date
1	To begin cleaning the Millipore TFF apparatus and Pellicon filter, repeat flushing of the unit with 500 ml water, as described in procedure 1.6.3. steps 1 through 14		
2	Disconnect the retentate tubing (silicone, translucent) from RET IN port and place in waste collection vessel. Place the end of the permeate tubing in the waste collection vessel.		
3	Open the retentate valve by turning it counterclockwise.		
4	Remove the reservoir cover and fill with 500 ml of 0.1N NaOH. Ensure the vent port is open by removing the plug from the VENT port and either leave open or install a Millex Filter.		
5	Open the tank outlet valve.		
6	Turn the pump on and increase the pump speed until the feed pressure gauge reads 20 psi. Check all system connections for leaks and tighten any connections as necessary.		
7	Continue pumping to the waste collection vessel until the level in the reservoir drops to 250 ml and then turn the pump off. Reconnect the retentate (silicone, translucent) tubing to the RET IN port.		
8	Connect the male luer end of the permeate tubing to the recirculation (DIA / RECIRC) port. Turn the pump on and increase the pump speed until the feed pressure gauge reads 20 psi. Check all system connections for leaks and tighten any connections as necessary.		
9	Adjust the retentate valve restriction by slowly turning the retentate valve clockwise until the retentate pressure gauge reads 10 psi. Adjust the pump speed and retentate valve restriction to achieve 30 psi feed pressure and 10 psi retentate pressure.		

Batch Record for Downstream Processing of Anti IL-8 mAb

10	Recirculate the cleaning solution for 30-60 minutes and then turn the pump off.		
11	To drain the system, disconnect the pump outlet (Sta-pure, white) tubing from the pump outlet port and place in waste collection vessel.		
12	Disconnect the retentate silicone (translucent) tubing from the RET IN port. Fluid should now drain by gravity. If additional drainage is required, a syringe can be placed on the end of the retentate tube and fluid can be blown down.		
13	Replace retentate tubing (silicone, translucent) in retentate port. Reconnect pump outlet tubing (Sta-Pure, white).		
14	Disconnect FEED IN tubing and place in collection vessel. Open tank outlet valve, turn pump speed up and let the reservoir drain.		
15	Stop the pump, close the outlet valve and Reconnect the pump outlet tubing (Sta-Pure, white) to the Feed In port.		
17	Repeat Flushing with MilliQ water as described above in Procedure 1.6.3. steps 1 through 14		

1.6.9 Pellicon XL Cassette Storage

#	Task		
1	Turn/loosen all of the lock nuts until you are able to remove the Pellicon XL cassette.		
2	Fill a 10 ml syringe with 0.05N NaOH Storage solution.		
3	Place the cassette in sink or tray that can contain any overflow. Attach the syringe to the retentate port and slowly push the solution into the device. Remove the syringe and replace all of the plugs on the ports and store flat at 4°C-25°C.		

1.6.10 Clean Base

#	Task		
1	Disconnect the power cord.		
2	Clean exterior surfaces, reservoir, and Labscale System Base with a mild soap and water solution.		

Batch Record for Downstream Processing of Anti IL-8 mAb

1.6.11. Attchments

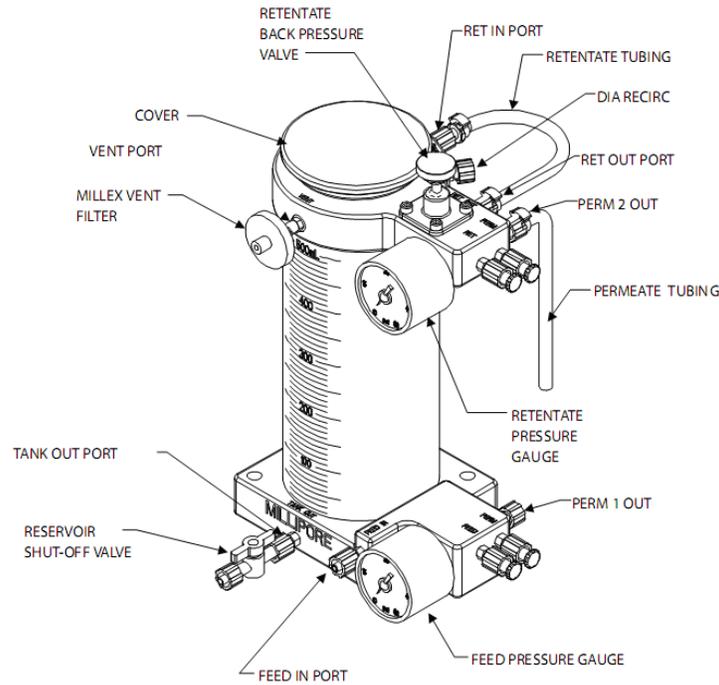


Figure 1: Reservoir Set Up

<http://www.millipore.com/userguides.nsf/docs/p60085>

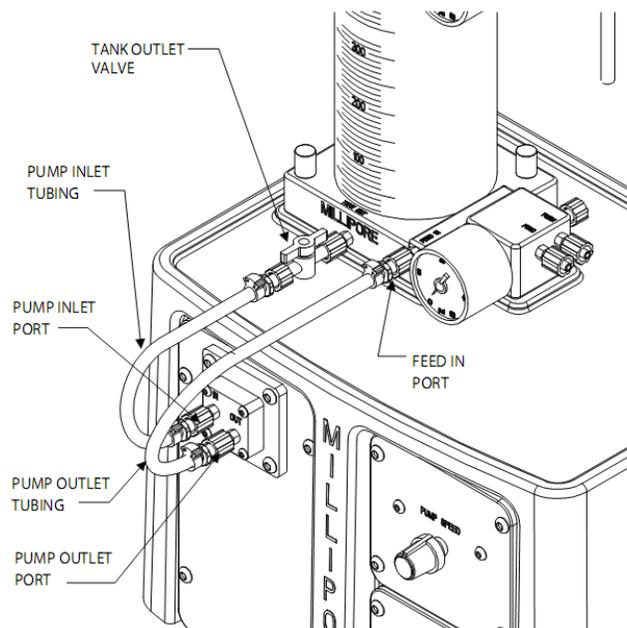


Figure 2: Pump Base Set Up

Batch Record for Downstream Processing of Anti IL-8 mAb

<http://www.millipore.com/userguides.nsf/docs/p60085>

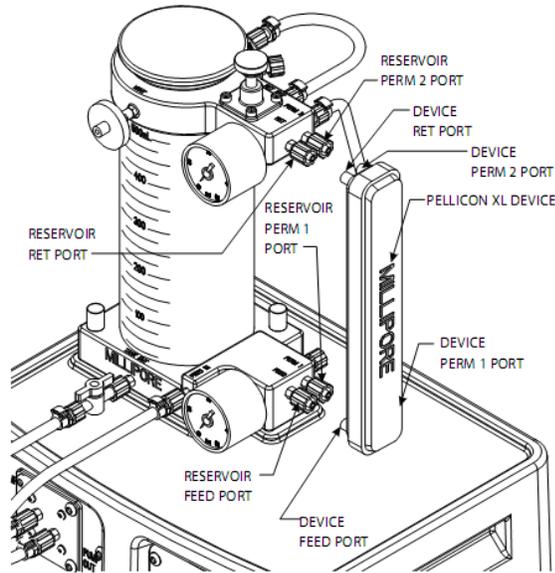


Figure 3: Installation of Pellicon XL Device

<http://www.millipore.com/userguides.nsf/docs/p60085>

Batch Record for Downstream Processing of Anti IL-8 mAb

2.0 Chromatography Operation:

2.1 Description

2.1.1. This batch record covers the precise operating steps necessary to purify recombinant mAb from concentrated conditioned cell culture medium using protein A affinity chromatography with the AKTA pure instrument.

2.2 Reference

<i>Title</i>	<i>Document Number</i>
SOP: Isolation of mAb (anti IL-8) from Conditioned Medium by Protein A Affinity Chromatography on the ÄKTApure Chromatography System	DP12
SOP: Operation of AKTA pure Chromatography System	DP 5
SOP: Bradford Protein Assay	
SOP: Quantification of CHO DP-12 Derived Anti IL-8 Monoclonal Antibody by ELISA	QCB

2.3. Equipment

<i>Equipment Type</i>	<i>Manufacturer, Model Number</i>	<i>Calibration Due Date</i>	<i>Initials/Date</i>	<i>Verifier/Date</i>
Chromatography System	GE Healthcare AKTApure 25			
Column	HiTrap Protein A-HP, 1ml Note: remove the column from 4°C storage and allow to come to room temperature	N/A		

2.4. Materials

<i>Component</i>	<i>Quantity Required</i>	<i>Quantity Used</i>	<i>Initials/Date</i>	<i>Verifier/Date</i>
Fraction tubes	30			
Syringe, 10 ml	2			
0.2 µm syringe filter	1			
Ehrlenmeyer flask, 125 ml	1			
Ehrlenmeyer flask, 500 ml	1			
50 ml conical tube	1			
0.2 µm vacuum filter unit	4			

Batch Record for Downstream Processing of Anti IL-8 mAb

2.5. Solutions

<i>Solution</i>	<i>ID</i>	<i>Date Prepared</i>	<i>Volume Required</i>	<i>Volume Used</i>	<i>Initials/Date</i>
Buffer A	20 mM sodium phosphate buffer, pH 7.0		500 ml		
Buffer B	0.1 M sodium citrate, pH 3.0		200 ml		
MiliQ water	Filtered, degassed MilliQ water		500 ml		
System Storage Solution	20% Ethanol		300 ml		
Neutralizer	1 M Tris base pH 9.0		100 ml		

2.6. Procedure

2.6.1. Preparation of Buffers and Solutions

#	<i>Task</i>	<i>Initials/Date</i>	<i>Verifier/Date</i>
Buffer A: Binding buffer: 20 mM sodium phosphate, pH 7.0			
1	Weigh 1.084 ± 0.02 g NaH ₂ PO ₄ and transfer to a 1200ml beaker with magnetic stir bar.		
2	Weigh 3.273 ± 0.02 g Na ₂ HPO ₄ and transfer to the same beaker.		
3	Measure 980ml MilliQ water in a graduated cylinder and add the water to the solids in the beaker.		
4	Stir until the solids have dissolved, check the pH, if needed adjust the pH with 1N phosphoric acid.		
5	Transfer to a 1L graduated cylinder and adjust the final volume to 1L.		
6	Sterile filter the solution, allowing it to degas for 15-20 minutes. Label appropriately.		
Buffer B: Elution buffer: 0.1M sodium citrate, pH 3.0			
1	Weigh 3.84g citric acid in a 400 ml beaker with magnetic stir bar.		
2	Dissolve in 180 ml MilliQ water.		
3	Adjust the pH dropwise with 10N NaOH, to a final pH of 3.0		

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4	Transfer the solution to a 250 ml graduated cylinder. Adjust the final volume to 200 ml		
5	Filter the solution, allowing it to degas for 15 – 20 minutes. Label appropriately		
	1M Tris base pH 9.0; neutralizer.		
1	Weigh 12.11g Tris base [tris(hydroxymethyl)aminomethane] into a plastic weigh boat and transfer to a 200 ml beaker with a stir bar.		
2	Measure 90 ml MilliQ water in a graduated cylinder and transfer the water to the beaker containing Tris powder. Stir until dissolved. Adjust the pH to 9.0 with 10M HCL		
3	Transfer the Tris solution quantitatively to a 100 ml graduated cylinder, rinsing the beaker with small aliquots of water, which are then added to the cylinder until a final volume of 100 ml is obtained.		
4	Filter the solution with a 0.22 µm filter. Degassing is not necessary.		

2.6.2. Chromatography system setup

#	Task	Initials/Date	Verified Initial/Date
1	Place or verify that Buffer A is in place, securely located atop the instrument. Insert tubing for inlet A1 to the bottom of the container. Approximate volume of Buffer A: _____ ml		
2	Place or verify that the Buffer B container is in place, securely located atop the instrument. Insert tubing for inlet B1 to the bottom of the container. Approximate volume of Buffer B: _____ ml		
3	Verify that the tubing labeled Outlet is placed into a 125 ml E. flask		
4	Verify that the Waste effluent tubing labeled W, W1, and W2, are placed in a 500 mL E flask		
5	Place an adequate supply of tubes (30), numbered sequentially, in the fraction collector carousel.		

Batch Record for Downstream Processing of Anti IL-8 mAb

6	Pipet 200 µl 1M Tris pH 9.0 into each tube in the carousel; ensure that the aliquot gets to the bottom of the tube (as opposed to clinging to the side).		
7	Rotate the tube carousel so that the #1 position is set to receive the initial drops. Lift the arm and swing it over to rest against the side of the first tube.		
8	Turn the AKTApure system on. The on/off switch is on the right side toward the rear of the instrument.		
9	Turn on the computer and login		
10	Open the Unicorn 6.3 software by: 1) double clicking the desktop icon 2) clicking 'OK' at the Log On-Unicorn dialog box		
11	Confirm that the installed column is a HiTrap Protein A-HP 1 ml (at room temperature).		

2.6.3. pH Electrode Calibration

#	Task	Initials/Date	Verified Initial/Date
1	Obtain three small beakers and pH standards for pH 4.01 and pH 7.0, as well as a 10 ml syringe and a bottle of MilliQ water.		
2	In the Unicorn System Control window, choose 'Calibration' from the System menu. From the drop down menu under 'Monitor to calibrate', select 'pH'.		
3	Click the 'Prepare for Calibration' button. You will hear the valve switch to the calibrate position.		
4	Follow the on-screen instructions for both pH standards. Enter the pH of the first pH standard buffer in the pH for buffer 1 field		
5	Fill a syringe with approximately 10 ml of the first pH standard buffer (pH 7). Connect the syringe to the Luer connector of pH valve port Cal , and inject the buffer. When the Current value is stable, click the Calibrate button.		
6	Thoroughly rinse the syringe with 3-4 changes of MilliQ water. Wash the pH flow cell by injecting water into pH valve port Cal .		
7	Enter the pH of the second pH standard buffer in the pH for buffer 2 field. Fill a syringe with approximately 10 ml of the second pH standard buffer. Connect the syringe to the Luer connector of pH valve port Cal , and inject the		

Batch Record for Downstream Processing of Anti IL-8 mAb

	buffer. When the <i>Current value</i> is stable, click the <i>Calibrate</i> button.		
8	The calibration date and time are displayed in the dialog, along with values for <i>Calibrated electrode slope</i> (should be $\geq 80\%$) and <i>Asymmetry potential at pH 7</i> (should be within the interval ± 60 mV. If the conditions are met, click the <i>Close</i> button to switch the pH valve back to the default position and to close the <i>Calibration</i> dialog.		

2.6.4 Column Equilibration

#	Task	Initials/Date	Verified Initial/Date
1	Equilibrate system and column as follows: 1) Navigate to the System Control window. 2) If the window is blank, choose menu item System\Connect to System and choose OK 2) In the File menu, select Open\Hi Trap Protein A 1ml Equilibration 3) Click Next until the Start button is shown, then choose it. 4) Allow the method to run to completion (about 15 minutes).		
2	Verify that eluent is directed into the waste flask		
3	Empty waste flask when the method is complete, then return it.		

2.6.5. Protein A Affinity Chromatography

Chromatographic run sequence summary:

- 1) Inject 9.5 ml from the Superloop; begin collecting 5 ml fractions; flow rate = 0.5 ml/min.
- 2) Wash unbound proteins through with up to 15 column volumes (CV) buffer A, until A280 stabilizes; collecting 2.5 ml fractions. Flow rate = 1 ml/min.
- 3) Elute bound immunoglobulins with step to 0.1M Na-citrate, pH 3; collecting 1 ml fractions and peak fractionation, for a total of 15 CV
- 4) Re-equilibrate column in buffer A until pH stabilizes; maximum 20 CV. Eluent to waste.

Batch Record for Downstream Processing of Anti IL-8 mAb

#	<i>Task</i>	<i>Initials/Date</i>	<i>Verified Initial/Date</i>
1	Obtain the concentrated Anti IL-8 mAb sample in the Buffer A collected from TFF. Sterile filter the sample using 10 ml syringe and 0.22 µm syringe filter in a 50 ml conical tube. Record the sample information Sample origin: Batch #: Date prepared: Volume:		
2	Sample injection into 10 ml Superloop: 1) Fill 10 ml syringe with filtered sample, being careful to avoid or eliminate any air bubbles 2) Dispense excess sample back into its original container, retaining 10+ ml in the syringe 3) Insert syringe firmly into sample inlet port with Luer lock tightened 4) Inject 10 ml Superloop		
3	Initiate the run: 1) Using the Unicorn 6.3 software, open the System Control window. 2) Under the File menu, choose Open\1ml Protein A Column ver2 3) In the resulting dialog box, input Sample Info into the designated cell. 4) Enter 5) Click Next (repeatedly) until the Start button is shown in the dialog box. 6) Click Start to begin the separation process.		
4	Upon completion, transfer the labeled tubes to a tube rack and store at 4°C for later analysis.		
5	Repeat the step 6.1 and 6.5 with remaining sample		

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2.6.6. Evaluate Chromatographic Separation

#	Task	Initials/Date	Verified Initial/Date
1	Open the chromatogram (will be the most recent one listed) in Unicorn "Evaluation" tool as follows: 1) In Unicorn 6.3 software, under the Tools menu, choose Evaluation. 2) In the Evaluation window, click the Results tab. 3) Find yours in the listed chromatograms, then double click to display it in the right frame.		
2	<i>Optional:</i> Customize chromatogram: 1)Open Customize tool 2)Accept the default, or select curves for UV, conductivity, fractions; 3)adjust Y axis values for optimum display of curves		
3	<i>Optional.</i> Determine protein content per fraction by Bradford Protein Estimation. Refer to the SOP for that procedure.		
4	<i>Optional</i> Use Operations\Fraction Histogram to indicate average protein content per fraction.		
5	<i>Optional.</i> Use Operations\Activity Histogram to enter <input type="checkbox"/> g amount per fraction, as determined using the ELISA or other analytical technique to determine specific Ab content.		
6	Save and Print: Save the chromatogram as a pdf: 1) While displaying finished chromatogram, choose File\Print 2) In the resulting dialog box, choose Preview 3) In the window that opens, click File\Save as PDF 4) Enter a name which refers to the sample, column and date (e.g. antiIL8 on HiTrap Protein A HP 09APR15) 5) Print a copy of the chromatogram for record keeping		
7	Save changes.		

Batch Record for Downstream Processing of Anti IL-8 mAb

3.0 History

<i>Revision Number</i>	<i>Effective Date</i>	<i>Preparer</i>	<i>Description of Change</i>
0	16APR16	Jason McMillan & Dr. David Frank	Initial release
1	18DEC18	Hetal Doshi	1.Changed the Pellicon XL cassette to 30,000 Molecular weight cut off 2. Buffer exchange step added.
2	10JAN20	Hetal Doshi	Combined Down stream batch record for TFF and chromatography operation into 1 document

SOP: ÄKTA pure Chromatography System Operation

Approvals

Preparer: Dr. David Frank
Reviewer: Jason McMillan
Reviewer: Hetal Doshi
Reviewer: Dr. Maggie Bryans

Date: 20APR16
Date: 21APR16
Date: 08JAN20
Date: 10JAN20

1. Purpose

1.1. This procedure describes the operation of the ÄKTA pure Chromatography System, controlled by Unicorn 6.3 software.

2. Scope and Applicability

2.1. Applies to chromatography of proteins, etc using a column installed on the GE ÄKTA pure Chromatography System and controlled by Unicorn 6.3 software.

3. Summary of Method

- 3.1. Method writing (programming)
- 3.2. Priming the pump rinsing system
- 3.3. Priming Inlets and purging pump heads
- 3.4. Assembly and installation of a 10 ml Superloop sample chamber.
- 3.5. Calibration of the pH electrode/detector.
- 3.6. Manual control of the instrument
- 3.7. Equilibration of system and column
- 3.8. Fraction collector setup
- 3.9. Application of sample
- 3.10. Washing and elution of column
- 3.11. Regeneration of system in preparation for subsequent run
- 3.12. Procedures for cleaning and short or long term storage of the system

4. References

- 4.1. Unicorn 6.3 Users Guide (electronic)
- 4.2. ÄKTA pure 25 Users Guide (electronic)
- 4.3. Manufacturer's literature/spec sheets for media
 - 4.3.1. HiTrap SP HP 5 information booklet (GE)
 - 4.3.2. L-Lysine HyperD reference sheet (Pall)
 - 4.3.3. Capto Q information booklet (GE)
 - 4.3.4. Ni²⁺ IMAC Profinity reference sheet (BioRad)
- 4.4. Batch Record for Downstream Processing of t-PA: TFF Operation

5. Definitions

5.1. N/A

6. Precautions

- 6.1. Routine care should be exercised in handling of buffers and samples of biological materials, which may have harmful biological activity in the case of accidental ingestion, needle stick, etc.
- 6.2. User should read and be familiar with general good practice as outlined in the ÄKTA pure Cue Cards located near the instrument.

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- 6.3. Avoid damaging the threads through the use of excessive force when connecting plastic fasteners.
- 6.4. Care must be taken to avoid air in the fluid path, which could damage the pumps or give spurious and uninterpretable readout from the UV and/or conductivity detectors.
- 6.5. Gloves and protective eyewear should be worn when handling samples and reagents (buffers), however it is preferable that the user remove gloves prior to entering commands via the computer keyboard or mouse.
- 6.6. Buffers must be degassed and filtered prior to use with the AKTA pure instrument. Samples should be centrifuged at 10000xg for 5 min before injection/introduction into the fluid path.
- 6.7. Equipment calibration check: The AKTA pure system calibration is automatic; baseline for measurements of A280 and conductivity are zeroed at the beginning of a chromatography run. Further adjustment is beyond the scope of this document and should be referred to a qualified technician.

7. Responsibilities

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

8. Equipment and Materials

- 8.1. AKTA pure chromatography system
- 8.2. Additional Lab Equipment: pH meter, balance
- 8.3. Lab Utensils: Beakers (250, 500ml), 500 ml graduated cylinders
- 8.4. Reagents: Filtered deionized water (MilliQ or similar), 20% ethanol
- 8.5. Lab Supplies: Filters (0.2 µm) and bottles for vacuum filtration and degassing of all chromatography buffers. Syringe (1ml). Tubes for fraction collector (3 ml – 15 ml capacity).
- 8.6. Column cleaning solution for HiTrap SP (GE) – 0.1M NaOH
- 8.7. Column cleaning solution for Lysine HyperD (Pall) – 0.1M NaOH;
- 8.8. Column cleaning solution for Canto-Q (GE) – 0.1M NaOH
- 8.9. Column cleaning solution for Ni²⁺ IMAC Profinity (Bio Rad) -

9. Procedure

- 9.1. Sample Collection and Preparation is beyond the scope of this document, however the system is compatible with a very broad range of biological samples, with the caveat that the sample must be free of debris or particulate matter. The operator will require 0.6 - 10 ml of sample per sample injection (see below). The goal (analytical vs preparative) of the chromatography will dictate the amount of sample needed and/or applied to the column. Concentration of the sample and buffer components/pH should be chosen to be compatible with the mode of separation.
- 9.2. Reagent Recommendations: Buffers should be prepared from the highest available grade reagents and water, filtered through a 0.2 µm filter and degassed thoroughly. Buffer preparation is beyond the scope of this document. Examples of buffers for specific applications where t-PA is the protein/biopharmaceutical of interest are:

SOP: ÄKTA pure Chromatography System Operation

9.2.1. HiTrap SP HP Cation exchange column:

9.2.1.1. Buffer A = 0.2M sodium acetate, pH 5.0, 0.01% Tween 80

9.2.1.2. Buffer B: 0.2M sodium acetate, pH 5.0, 0.01% Tween 80, 1M NaCl

9.2.2 L-Lysine Ceramic HyperD Affinity column:

Buffer A: 20mM sodium phosphate, pH 7.5, 0.01% Tween 80

Buffer B: 20mM sodium phosphate, pH 7.5, 0.01% Tween 80, 1M NaCl

Elution Buffer B2: 20mM sodium phosphate, pH 7.5, 0.01% Tween 80, 0.2M arginine hydrochloride

9.2.3 Ni²⁺-IMAC column

Buffer A: 20mM sodium phosphate, pH 7.0, 0.5M NaCl 0.01% Tween 80

Buffer B: 20mM sodium phosphate, pH 7.0, 0.5M NaCl, 0.01% Tween 80, 0.5M imidazole

9.2.4 Protein-A Sepharose column

Buffer A: 20 mM sodium phosphate, pH 7.0

Buffer B: 0.1 M sodium citrate, pH 3.0

9.3. Method writing in Unicorn 6.3.

For the Unicorn 6.3 software to be able to control the AKTA pure instrument, it is necessary to assemble programmed steps into a method. The steps vary for a given column and protein of interest, but typically include Sample Loading, Column Washing, Elution, and Equilibration. Often more steps are required. In most situations, the method will be written and stored in the Method Navigator within Method Editor; refer to the Batch Process Record or Process SOP for the protein of interest to find the name of the specific method. In the event a new or modified method is required, here are the (minimum) steps needed.

9.3.1. In the Unicorn software, navigate to the Method Editor window.

9.3.2. Under the File menu, choose New Method.

9.3.3. In the pop-up window, choose a pre-defined method for the type of chromatography you will be doing, then click OK.

9.3.4. Each step in the program is referred to as a 'Phase' in the software. The phases for the predefined method will appear in the center pane of the Method Editor window.

9.3.5. Click the Method Settings phase to display its properties in the right hand pane. If you are using a GE Life Sciences prepacked column, find and select it in the Column Type dropdown menu. Its properties and suggested flow rate will automatically be chosen. If your column is not listed, enter the Column Volume, Pressure Limit and Flow Rate (specified by the manufacturer) in their respective boxes. Choose Inlets, usually A1 and B1; these can be specified for each step (phase) as you progress through method writing.

9.3.6. While still in the Method Settings pane, click the Start Protocol button. A pop-up window appears that allows the operator to choose items that will be presented for operator's information/approval when a run of the method is initiated. Check the boxes for Method Information (which will show the expected volume and time required, allowing one to check that the pumps will not run dry due to inadequate supply of buffer), and for Questions. Click the button labeled Define Questions. This allows the operator to specify useful information that must be entered before

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the run can start, which may include sample characteristics, buffer composition/preparation date, operator, etc. Follow the prompts to enter questions, check the Mandatory and Display in Chromatogram boxes, then Preview to confirm the appearance.

- 9.3.7. Click the button for Equilibration phase in the center pane to display its properties in the right pane. Confirm that 'Reset UV monitor' is checked. Check 'Fill the system with the selected buffer' to quickly flush inappropriate buffers between the pumps and the injection valve.
- 9.3.8. Click the Sample Application phase in the center pane to display the parameters.
 - 9.3.8.1. Uncheck the box 'Use the same flow rate as in Method Settings' – typically a capture method such as ion exchange or affinity separations achieves higher binding efficiency at lower flow rates.
 - 9.3.8.2. Select the Loop type and enter the sample volume to be injected. In the 'Empty loop with' box, enter a volume sufficient to flush all sample from the loop.
 - 9.3.8.3. Confirm that the correct inlet lines are specified; most methods use A1 and B1.
 - 9.3.8.4. If you suspect that your sample will exceed the capacity of the column, check the 'Interrupt sample application at UV' and enter a value (which will likely have to be determined empirically). A better approach is to limit the quantity of sample applied so that the operator is confident that the capacity of the column will be adequate to the task.
 - 9.3.8.5. In the 'Fractionate' section, choose the path that the eluent should follow. It is customary to collect the unbound protein in a container ('using outlet valve' directs the flow-thru to the tube labeled 'Out'; place it in a clean flask) or in fractions ('using fraction collector').
 - 9.3.8.6. Fractionation settings: choose 'Fixed outlet' using outlet valve. Choose 'fixed volume fractionation' to collect the flow-thru in constant volume fractions with the fraction collector.
- 9.3.9. Click the Column Wash phase button in the center pane.
 - 9.3.9.1. Check the box to 'Use the same flow rate as in Method Settings'.
 - 9.3.9.2. Confirm the inlets for A and B, and that the 'Fill the system....' box is unchecked.
 - 9.3.9.3. Select a value for 'Wash until', which should be at least 10 column volumes (for the small columns used in this course). Select the radio button for 'the following condition is met', choose Stable UV, set the Accepted UV fluctuation to 0.1mAU (experience may dictate a change, so revisit this setting on occasion), and set the Maximum wash volume to 20 CV.
 - 9.3.9.4. Fractionate: column wash is diverted 'in waste (do not collect)'.
- 9.3.10. Click the Elution button in the center pane and make these settings:
 - 9.3.10.1. Check the box for 'Use the same flow rate as in Method Settings'
 - 9.3.10.2. Confirm Inlet A and B specify the tubing that is placed in buffers A and B.
 - 9.3.10.3. Gradient elution is the default selection.
 - 9.3.10.3.1. Choose the 'Start at' concentration of buffer B, usually 0%. If greater than 0%, check the 'Fill the system....' box.

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9.3.10.3.2. Specify the Type of gradient (step or linear), target % B and total volume (recommend 10 CV initially) for the gradient in this segment.

9.3.10.3.3. Add more segments as needed. Commonly a final elution of 2-3 CV at high strength eluting conditions (e.g. 1M NaCl for an ion exchange separation) is included.

9.3.10.3.4. Fractionate settings should be for 'using fraction collector' and 'fixed volume fractionation'. Set the Fixed fractionation volume to a volume that represents about 1/20 of the total gradient volume.

9.3.11. Click the (second) Equilibration button to set parameters for re-equilibration of the column for subsequent runs.

9.3.11.1. Confirm that the 'Reset UV Monitor' box is unchecked.

9.3.11.2. Check the 'Use the same flow rate....' box.

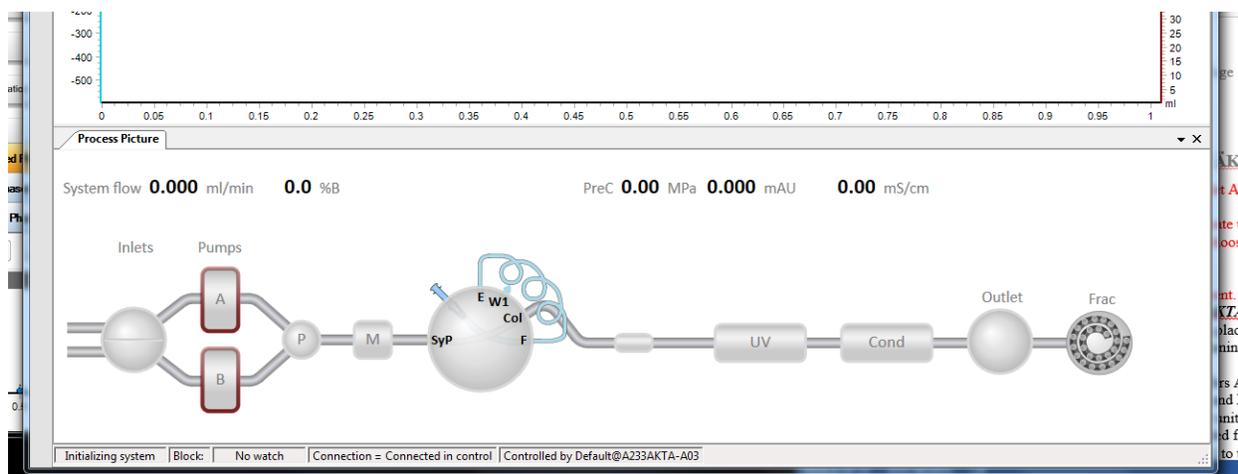
9.3.11.3. Confirm that Inlet A and B are correct and correspond to the buffers A and B.

9.3.11.4. Set the 'Equilibrate until' volume to 5 CV.

9.3.12. Under the File menu, choose Save and name the method uniquely; select the correct folder and Save.

9.4. Manual Control of the instrument.

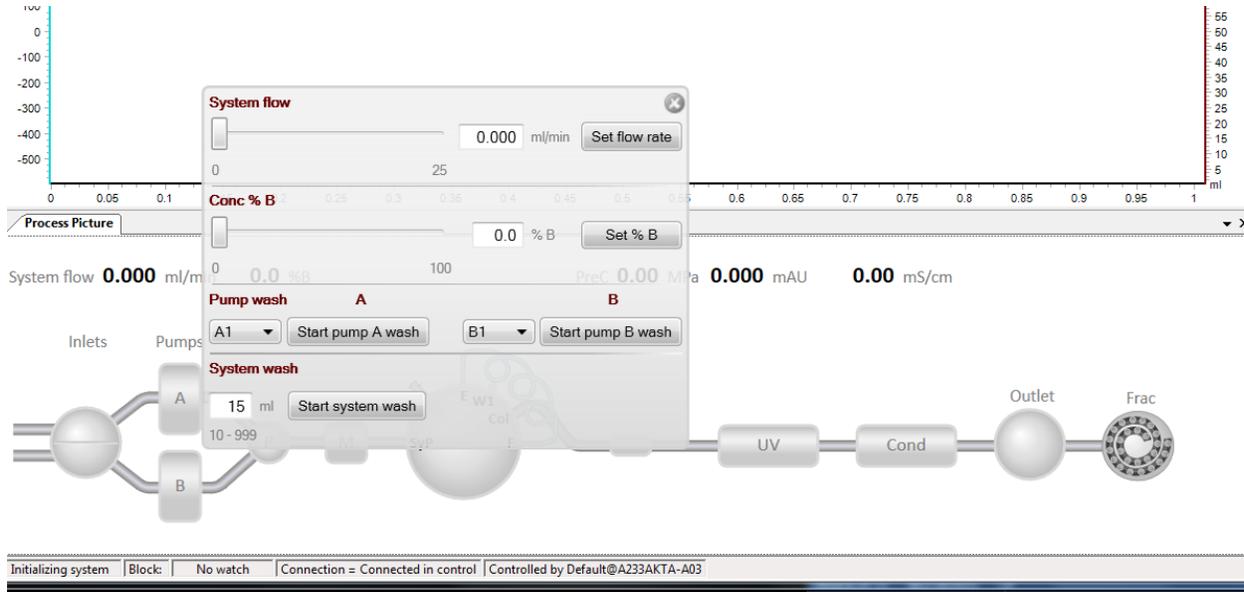
The AKTA pure instrument may be controlled 'manually' via the System Control window lower pane graphic interface (or 'Process Picture'), as pictured here.



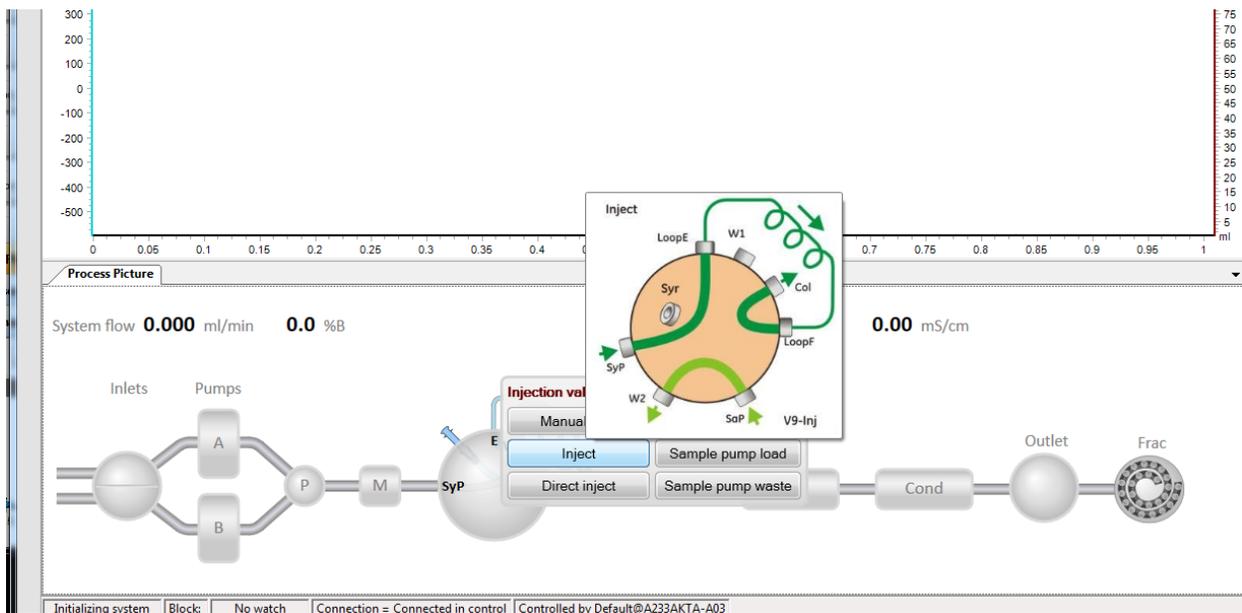
9.4.1. A control panel that allows selection of inlet pumps/lines A1, A2 B1 And B2 will appear when one clicks on the small bisected circle on the left side.

9.4.2. By clicking on either Pump A or Pump B in the depiction, you can access a dialog box which allows for quick setting of flow rate and buffer composition, as well as convenient pump wash features as shown:

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9.4.3. The large circle (approximately the center of components) in the diagram represents the sample injection valve. Upon clicking it, one will see a panel of 6 options for the position of the valve (five are visible in the diagram below). Hovering over each option causes a new diagram to appear that shows the flow path through the valve for that particular selection; shown here is the Inject position, with flow coming from the system pumps (SyP), going through LoopE valve inlet, carrying contents of the sample loop out through LoopF valve outlet and onto the column (Col).



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- 9.4.4. Clicking the UV detector icon reveals a command to 'Auto Zero UV'.
- 9.4.5. The small circle to the right is the Outlet valve. By clicking on it with the electronic mouse pointing device, the operator may select whether the eluent goes to Waste, the Outlet tubing, or the Fraction Collector.
- 9.4.6. Various parameters of the current run condition are displayed directly above the manual control interface. Pay particular attention to pre-column pressure (PreC x.xx MPa) to avoid over pressurizing/damaging the column. Most of our columns have a limit of 0.5 MPa.
- 9.5. Priming the pump rinsing system. The pump piston rinsing system protects the seal that prevents leakage between the pump chamber and the drive mechanism of the pump. Priming of the pump rinsing system is done before the run
 - 9.5.1. Remove the pump rinsing liquid tube from the holder located on the right-hand bottom corner of the system.
 - 9.5.2. Fill the pump rinsing liquid tube with 50ml of 20% ethanol
 - 9.5.3. Place the pump rinsing liquid tube back in the holder
 - 9.5.4. Insert the inlet tubing to the system pump piston rinsing system in the rinsing solution tube. (Note: Make sure that the inlet tubing reaches close to the bottom of the rinsing solution tube)
 - 9.5.5. Connect 25 to 30 ml syringe to the outlet tubing of the system pump piston rinsing system. Draw liquid slowly into the syringe.
 - 9.5.6. Disconnect the syringe and discard its contents.
 - 9.5.7. Fill the rinsing solution tube so that the tube contains 50 ml of 20% ethanol.
- 9.6. Prime inlets and purge pump heads. Before using the system pumps it is important to: Prime the inlets (fill the buffer inlets with liquid) and to Purge the system pumps (remove air from the pump heads)
 - 9.6.1. Prime the Inlets.
 - 9.6.1.1. Make sure that all inlet tubing that is to be used during the method run is placed in the correct buffer.
 - 9.6.1.2. Turn on the AKTA pure system.
 - 9.6.1.3. Open the unicorn 6.3 software
 - 9.6.1.4. Open the **system control** module in the unicorn 6.3 software
 - 9.6.1.5. In the **Process Picture** click on the buffer inlets
 - 9.6.1.6. Select the position of the inlet to be filled. Select the positions in reverse alphabetical order and start with the highest number. For example, if all four inlets are to be filled, fill them in the following order: B2, B1, A2, and A1. The inlet valve switches to the selected port.
 - 9.6.1.7. Connect 10 ml syringe to the purge valve of right pump head of the pump system B. Make sure that the syringe fits tightly
 - 9.6.1.8. Open the purge valve by turning it counterclockwise about three quarters of a turn. Draw liquid slowly into the syringe until liquid reaches the pump and no air bubbles are visible in the line.

SOP: ÄKTA pure Chromatography System Operation

- 9.6.1.9. Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.
- 9.6.1.10. Repeat step 9.6.1.6. to 9.6.1.9. for each piece of inlet tubing and their respective pump heads. For example, when priming inlet B1 select left pump head of the pump system B.
- 9.6.2. Purge System pump B
 - 9.6.2.1. Make sure that the piece of waste tubing connected to the injection valve port W1 is placed in a waste vessel
 - 9.6.2.2. In the **Process Picture** click on the **injection valve** and select **System pump waste**. The injection valve switches to waste position. This is necessary to achieve a low back pressure during purge procedure.
 - 9.6.2.3. In the **Process Picture** click on the pumps
 - 9.6.2.4. Set **Conc % B** to 100% B and click **Set % B**. only system pump B is active
 - 9.6.2.5. In the **Process Picture** click on the buffer inlets B2. The inlet valve switches to the selected port.
 - 9.6.2.6. In the **Process Picture** click on the pumps
 - 9.6.2.7. Set the **System flow** to 1.0 ml/min. Click **Set flow rate**. A system flow starts
 - 9.6.2.8. Connect a 10 ml syringe to the purge valve of the left pump head of system pump B. Make sure that the syringe fits tightly into the purge connector.
 - 9.6.2.9. Open the purge valve by turning it counterclockwise about three quarters of a turn. Draw a small volume of liquid slowly into syringe.
 - 9.6.2.10. Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.
 - 9.6.2.11. Connect the syringe to the purge valve on the right pump head of System pump B, and repeat step 9.6.2.8 to 9.6.2.10. Keep the system flow running.
- 9.6.3. Validate purge of pump B
 - 9.6.3.1. In the **Process Picture** click on the **Injection valve** and select **Manual Load**.
 - 9.6.3.2. Make sure the pump flow is on.
 - 9.6.3.3. In the **Chromatogram** pane check the **PreC pressure**. If the PreC pressure does not stabilize within a few minutes there may be air left in the pump. Refer AKTA pure system handbook for a troubleshooting guide.
- 9.6.4. Purge System pump A
 - 9.6.4.1. Purge both pump heads of pump system A by repeating step 9.6.2.1. to 9.6.2.11. but replace Set **Conc % B** to 0% and click **Set % B** in step 9.6.2.4.
- 9.6.5. Validate purge of pump A
 - 9.6.5.1. Repeat step 9.6.3.1. to step 9.6.3.3.
- 9.7. Preparation and installation of Superloop 10 sample injection device. The Superloop is stored disassembled and requires assembly and filling with buffer prior to installation on the AKTA pure instrument, as described here. Refer to diagram XXX. Gloves should be worn during handling of the Superloop parts.
 - 9.7.1. Rinse/wet O-rings on the end pieces and movable seal with deionized water.
 - 9.7.2. Insert the movable seal into the graduated glass tube from the bottom (zero) end. Observe proper orientation of the seal; the end with the O-ring should be closest to

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the bottom. Using a glass rod with smooth end or a plastic pipette, push the seal into the tube until the O-ring is between the 1ml and 2 ml graduations.

- 9.7.3. Mount the glass tube on a lab stand with clamp. Working over a sink or container to catch any overflow, pipet enough buffer A into the upper portion of the tube to fill it.
- 9.7.4. Mind the liquid that will squirt from the tubing; direct it into the sink. Insert one of the inner end pieces (with tubing attached) into the glass tube, contacting the liquid meniscus to eliminate air bubble entrapment. Press the end piece completely into the glass tube.
- 9.7.5. Invert the tube in the clamp/support and wet the movable seal with a small amount of 20% EtOH (or buffer A if it contains a detergent). It may be necessary to use the pipet to eject any air bubbles that stubbornly adhere to the glass and/or movable seal. When bubbles have been eliminated, completely fill the tube with buffer A. Minding the liquid that will squirt from the tubing, insert the remaining inner end piece with tubing attached.
- 9.7.6. Rotate the bottom inner end piece so that the slotted end (inside the glass tube) aligns with the small notch inside the glass tube. This alignment is important to establish and maintain; otherwise backpressure in the pumps could increase and prevent completion of the run.
- 9.7.7. Remove the glass tube with end pieces from the clamp.
- 9.7.8. Attach the bottom outer end piece by threading it onto the glass tube.
- 9.7.9. Slide the plastic protective jacket over the glass tube and seat it firmly into the bottom outer end piece.
- 9.7.10. Attach the top outer end piece to the remaining exposed threaded end of the glass tube.
- 9.7.11. To install the assembled Superloop 10 onto the ÄKTA pure instrument, place the lab support and clamp near the instrument on the left side, then mount the Superloop in the clamp. Adjust clamp vertically and horizontally as needed to place the Superloop in close proximity to the injection valve.
- 9.7.12. Attach the tubing on the top of the Superloop to the injection valve port labeled 'loop E' using the threaded connector. Confirm that the tubing is firmly attached and will not easily pull out of the fitting.
- 9.7.13. By default, the injection valve should be in the 'Manual Load' position upon booting up the instrument. Using the manual control feature in the Unicorn software, confirm that the valve is in Manual Load position. If not, switch the valve to the correct position by clicking the injection valve on the system control diagram, then selecting 'Manual Load'.
- 9.7.14. Fill a 1 ml syringe with buffer A, attach it to the injection port and inject a small volume of buffer, so that liquid just fills the valve port labeled 'loop F'.
- 9.7.15. Attach the bottom Superloop tubing to the injection valve port labeled 'loop F' using the threaded connector. Confirm that the tubing is firmly attached and will not easily pull out of the fitting.

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- 9.7.16. Attach the top Superloop tubing to the injection valve port labeled ‘loop E’ using the threaded connector. Confirm that the tubing is firmly attached and will not easily pull out of the fitting.
- 9.7.17. The Superloop is now ready to be flushed (see section 9.6) and prepared for sample injection.
- 9.8. ***Filling the Superloop with Buffer A.*** This step is important to ensure that the sample chamber and backside of the loop are equilibrated with the starting buffer for a given chromatographic run. Most of the residual ethanol and other compounds in the injection valve flow path will be expelled in the process.
- 9.8.1. Place degassed Buffer A atop the instrument and insert inlet tubing A1 into the buffer container, ensuring that the filter rests on the bottom of the container.
- 9.8.2. Use the pump priming syringe connected to pump A1 to draw 10 ml of buffer A through the inlet A1 tubing and close the priming valve. Using the manual control panel in the System Control window, click on Pump A in the diagram and select “Pump A Wash”.
- 9.8.3. Upon completion of the wash, set the flow rate to 1 ml/min. Click on the injection valve depiction and select “Inject”.
- 9.8.4. When the Superloop movable seal arrives at the zero position, change the injection valve position to ‘Manual Load’. Allow the pump to continue at 1 ml/min.
- 9.8.5. Fill a 10 ml syringe with buffer A and inject sufficient volume to completely fill the Superloop. See section 9.10 for more information on sample injection.
- 9.8.6. Once again, change the injection valve position to ‘Inject’ using the manual control feature of the software interface. Increase the flow rate to 2 ml/min.
- 9.8.7. When the Superloop movable seal is at the zero position, stop the pump. Click the ‘Stop’ icon (a solid square) in the toolbar near the top of the System Control window.
- 9.9. Start-up and preparation of AKTA pure Instrument and computer:
Degassed buffers should be in place prior to turning on the AKTA pure instrument. Equipment start-up requires turning on the instrument and, separately, the computer connected to it.
- 9.9.1. Place the degassed buffers A and B on top of the AKTA pure instrument.
- 9.9.2. Locate Inlet tubing A1 and B1 (atop the instrument and resting in water or 20% ethanol). Each has a filter unit attached, which distinguishes them from A2 and B2; those end in a male threaded fitting and will not be used for a two-buffer procedure.
- 9.9.3. Transfer tubing Inlet A1 to the buffer A bottle.
- 9.9.4. Transfer tubing Inlet B1 to the buffer B bottle.
- 9.9.5. The On/Off switch for the instrument is located on the right side toward the rear of the housing. Switch to the ‘On’ position. Audible emanations from within the instrument cabinet indicate that the AKTA pure system is going through its brief initialization sequence.
- 9.9.6. The computer On/Off switch is located on the front of the Dell desktop computer unit, near the top of the case. Press in to turn on the computer.
- 9.9.7. Login to the computer using credentials provided by the College.

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- 9.9.8. Double click the Unicorn 6.3 icon on the desktop to open the software which controls the instrument functions. Click OK in the “Log In – Unicorn” dialog box that appears.
- 9.9.9. Open the System Control window (under Tools menu, if not opened automatically on startup).
- 9.9.10. The top pane of the window will show the current state of the instrument, and the bottom pane shows the fluid path and manual controls. If the window is blank, go to the System menu and select Connect to Systems, check the box by AKTA pure 25 and click OK.
- 9.9.11. Confirm that the correct column is attached to the system. If not, refer to Section 9.4 (Installing/Changing a Chromatography Column on the AKTA pure Chromatography System).
- 9.9.12. Under the File menu, choose Open and select the method specified in the process SOP
- 9.9.13. A dialog box appears that allows the method to be started. Click Start to initiate flushing of the pumps and equilibration of the column.
- 9.9.14. While the equilibration method is running, prepare the fraction collector for later steps by filling the carousel with clean tubes.
- 9.9.15. Allow the program to run to completion, about 15 minutes.
- 9.10. Calibration of the pH Electrode
Calibration of the pH detector is performed daily, when the instrument is in use. The calibration procedure utilizes ordinary pH standards found in the lab. Calibration is dictated by a method built into Unicorn.
 - 9.10.1. Obtain three small beakers and pH standards for pH 4.01 and pH 7.0, as well as a 10 ml syringe and a bottle of MilliQ water.
 - 9.10.2. In the Unicorn System Control window, choose ‘Calibration’ from the System menu. From the drop-down menu under ‘Monitor to calibrate’, select ‘pH’.
 - 9.10.3. Click the ‘Prepare for Calibration’ button. You will hear the valve switch to the calibrate position.
 - 9.10.4. Follow the on-screen instructions for both pH standards. Enter the pH of the first pH standard buffer in the *pH for buffer 1* field.
 - 9.10.5. Fill a syringe with approximately 10 ml of the first pH standard buffer (pH 7). Connect the syringe to the Luer connector of pH valve port **Cal** and inject the buffer. When the *Current value* is stable, click the *Calibrate* button.
 - 9.10.6. Thoroughly rinse the syringe with 3-4 changes of MilliQ water. Wash the pH flow cell by injecting water into pH valve port **Cal**.
 - 9.10.7. Enter the pH of the second pH standard buffer in the *pH for buffer 2* field. Fill a syringe with approximately 10 ml of the second pH standard buffer. Connect the syringe to the Luer connector of pH valve port **Cal** and inject the buffer. When the *Current value* is stable, click the *Calibrate* button.
 - 9.10.8. The calibration date and time are displayed in the dialog, along with values for *Calibrated electrode slope* (should be $\geq 80\%$) and *Asymmetry potential at pH 7* (should be within the interval ± 60 mV. If the conditions are met, click the *Close*

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button to switch the pH valve back to the default position and to close the **Calibration** dialog.

- 9.10.9. If values for the slope and potential are not within acceptable values, clean the pH electrode and repeat the calibration procedure. If this does not help, replace the electrode.
- 9.11. Installing/Changing a Chromatography Column on the AKTA pure Chromatography System.

It is imperative that the following operations be performed in such a way as to prevent the introduction of air bubbles into the column and fluid path, which is achieved by making liquid-to-liquid (drop-to-drop) contact prior to inserting the threaded fitting into its position.

 - 9.11.1. Have on hand a few paper lab towels and a 250 ml beaker to catch waste.
 - 9.11.2. Remove tube connector from the UV detector inlet by unscrewing the knurled fastener.
 - 9.11.3. Initiate flow manually at 0.5 ml/min collecting waste in the beaker or towel.
 - 9.11.4. Remove the plug from the column inlet and place a few drops of 20% ethanol in the inlet, filling it to insure the absence of air. Also add drops of 20% ethanol to the UV detector inlet.
 - 9.11.5. As a droplet emerges from the inlet tubing, touch it to the liquid in the column inlet and begin to thread the fitting in, leaving slight looseness of threads so that liquid escapes around the fitting and pressure buildup in the column is prevented.
 - 9.11.6. Remove the column bottom plug and screw the column directly into the UV detector inlet.
 - 9.11.7. Tighten the column inlet fitting just enough to prevent leaking.
 - 9.11.8. The column is now ready to equilibrate in buffer (step 9.3.12) prior to performing a chromatography run.
- 9.12. How to fill a sample loop.
 - 9.12.1. Fill an appropriately sized syringe with sample, taking care to remove bubbles.
 - 9.12.2. Connect the syringe to the injection port (see Fig. 4).
 - 9.12.3. Open the System Control module in the Unicorn software.
 - 9.12.4. In the Process Picture click the Injection Valve and select Manual Load to confirm that the valve is in, or switch the valve to, manual load position.
 - 9.12.5. Depress the syringe plunger to inject sample into the sample loop, and leave the syringe attached to the port (prevents sample loss due to siphoning). Regarding amount to inject, if sample preservation is a consideration, inject only enough to fill the loop. If sample is abundant and/or relatively easy to generate, inject about twice the loop volume to insure complete filling.
- 9.13. Performing a chromatography run:
 - 9.13.1. Place the fraction collector tube 1 near the outlet tubing from the instrument (refer to attachment Fig 1) so that it will touch the arrow on the white paddle of the fraction collector arm. Note: To rotate the carousel, reach around the left side of the collector to find a rubber roller pressing against the carousel (Fig 2). Pull the roller away from the carousel (Fig. 3); the carousel will rotate freely as long as the roller is held. When the first tube is in the correct position, release the roller.

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- 9.13.2. Gently raise the arm and swing it into position against tube 1.
- 9.13.3. Place all 'Waste' tubing, labeled W, W1 & W2 in 1 L Erlenmeyer flask.
- 9.13.4. Place the tube labeled Out in a 125 ml Erlenmeyer flask.
- 9.13.5. Using a 1 ml syringe, aspirate 0.6 ml of the tPA sample into the syringe, expel any bubbles and insert the loaded syringe into the injection port.
- 9.13.6. Inject the sample into the port to fill the 0.5 ml sample loop.
- 9.13.7. Open the Unicorn software and navigate to the System Control window.
- 9.13.8. Under the File menu, choose Open and select the method with file name "*HiTrapSP tPA Production*".
- 9.13.9. In the dialog box that opens, enter operator's name, sample notes.
- 9.13.10. Click Next; note the time and volume for the run; make sure there is excess buffer A and B.
- 9.13.11. Click Next. Record the buffer composition of each buffer and the sample identity.
- 9.13.12. Click Next. Enter a filename composed of the method name, date, operator or group initials, for example "*HiTrapSP tPA 16May15 CertGroup*".
- 9.13.13. Click Start. The instrument should begin to execute the method, as evidenced by a soothing hum from the pumps and drops of liquid falling into tube 1 from the fraction collector outlet.
- 9.13.14. Observe that the fraction collector is receiving drops.
- 9.13.15. Monitor the computer screen for error messages or warnings.
- 9.13.16. Allow the method to run to completion, at which time the system will be re-equilibrated and ready for subsequent runs by repeating section 9.4.
- 9.14. Equipment shut-down and short term (less than 3 days) storage
 - 9.14.1. After completion of the final separation of the day, transfer Inlet tubing A1 and B1 to a flask of degassed Milli-Q water (250 ml or greater).
 - 9.14.2. In the Unicorn software, open the System Control window.
 - 9.14.3. Under the File menu, choose Open, then select the method 'System Short Term Storage'.
 - 9.14.4. Click Start.
 - 9.14.5. Allow the method to run to completion, as indicated by an audible tone and onscreen window.
 - 9.14.6. Place the pH valve in the 'Calibration' position (System Control window; System\Calibrate menu). Fill a 10 ml syringe with pH electrode storage solution and inject 9 ml it into the calibration port. Leave the syringe attached.
- 9.15. Turn off the instrument or perform the long term storage routine as needed (section 9.6).
- 9.16. Cleaning of the Superloop 10 sample holder- short term.
 - 9.16.1. For short term storage of the Superloop on the AKTA instrument, inject 2 ml Milli-Q water into the sample chamber.
 - 9.16.2. Pump it out to waste by temporarily disconnecting the outlet tubing that is connected to the injection valve at port 'loop F'.

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- 9.16.3. Using manual control in the System Control window of Unicorn, set the flow rate to 2 ml/min and the injection valve position to Inject. Allow pump A to run until the Superloop chamber is empty.
- 9.16.4. Reconnect to 'loop F'.
- 9.16.5. Repeat steps 9.6.1 thru 9.6.4 three times.
- 9.16.6. Inject 10 ml water into the sample chamber of the Superloop.
- 9.17. Equipment shut-down and long term (3 days or more) storage
 - 9.17.1. After completion of the System Short Term Storage method, transfer Inlet tubing A1 and B1 to a flask of degassed 20% ethanol (250 ml or greater).
 - 9.17.2. In the Unicorn software, open the System Control window.
 - 9.17.3. Confirm that the pH valve is in the 'Bypass' or 'Restrictor' position.
 - 9.17.4. Under the File menu, choose Open, then select the method 'System Long Term Storage'.
 - 9.17.5. Click Start.
 - 9.17.6. Allow the method to run to completion, as indicated by an audible tone and onscreen window.
 - 9.17.7. Turn off the instrument.
 - 9.17.8. Remove the Superloop from the instrument and carefully disassemble it. Handwash all parts with a general-purpose lab cleaner, rinse well and allow to air dry. Store the dried components in their original box.
- 9.18. Cleaning the system
 - 9.18.1. Column Removal – have paper towels on hand to catch drips. You will also need an appropriate plug for each end of the column.
 - 9.18.1.1. Use the Manual Control in the System Control window to set the flow rate of buffer or water to 0.5 ml/min.
 - 9.18.1.2. Disconnect the column outlet from the UV detector and fit the connector with a plug, slowly so that emerging droplets displace *all* air from the plug. Leave the plug slightly loose so as to prevent backpressure buildup.
 - 9.18.1.3. Slowly disconnect the tubing from the column inlet, allowing liquid to fill the cavity where the connector attaches. Attach the tubing directly to the UV detector inlet and stop the flow of buffer/water.
 - 9.18.1.4. Completely tighten the column outlet plug but be careful not to overtighten and strip the threads.
 - 9.18.1.5. Carefully insert a plug into the column inlet threads, displacing liquid but not allowing air to enter.
 - 9.18.1.6. The column may now be stored.
 - 9.18.2. Minimal Cleaning – After every day of use, perform short-term storage (9.10).
 - 9.18.3. Thorough Cleaning – Should be performed weekly.
 - 9.18.3.1. Remove the column (9.12.1) from the system prior to thorough cleaning of the system with 0.5M NaOH.
 - 9.18.3.2. Immerse all pump inlet tubes in a container of 0.5M NaOH.
 - 9.18.3.3. **Important:** Switch the pH electrode valve to 'Bypass' or 'Restrictor' position to prevent damage to the electrode.
 - 9.18.3.4. Run the method 'System Clean'.

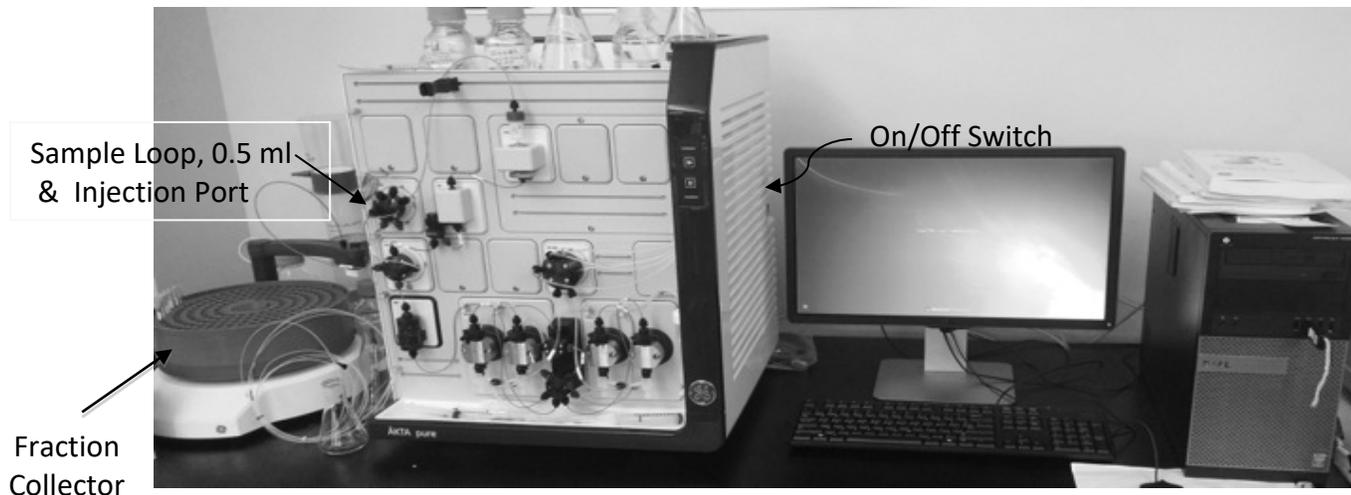
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- 9.18.3.5. Remove the pump inlet tubing from the NaOH container, rinse each carefully with a squirt bottle of MilliQ water and place in a flask of filtered and degassed MilliQ water.
- 9.18.3.6. Run the method 'System Short Term Storage'.
- 9.19. Chromatogram printout
 - 9.19.1. In the Unicorn software interface, open the Evaluation window.
 - 9.19.2. In the Result Navigator pane, click the Results tab.
 - 9.19.3. Locate the file of interest and double click its name to display your chromatogram in the right pane.
 - 9.19.4. *Optional:* Click the Customize button to open a dialog box that allows you to specify what curves display and the scale of each axis. Recommended are the UV Chrom curve, Conductivity, and Fraction Number.
 - 9.19.5. Click the Report button, check the Default report in the selection window and click Preview.
 - 9.19.6. Under File, choose to Print (or Save as PDF to use a different printer).

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10. Attachments/Figures

Fig. 1. Diagram of AKTA pure instrument, fraction collector and computer



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Fig. 2. *ÄKTA pure Instrument Features*

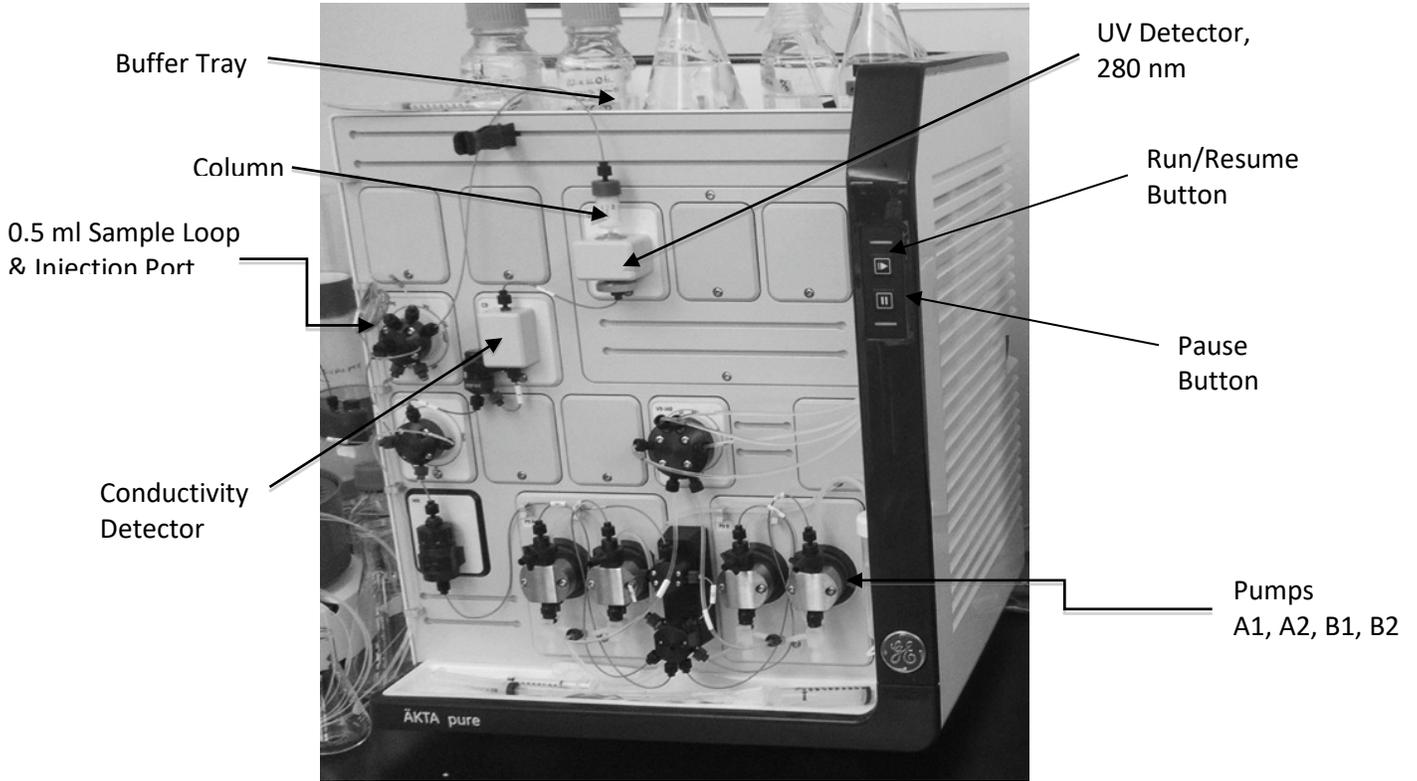


Fig 3. *System Control window within the Unicorn 6.3 software.*



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Fig. 4. Detail of Injection Port with Syringe in Place.

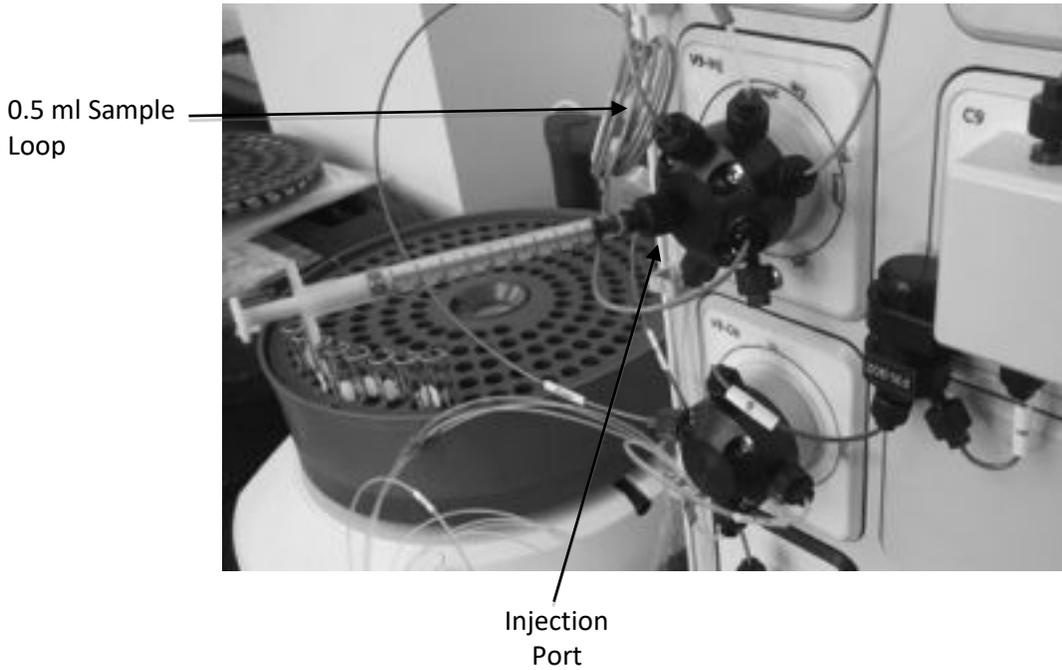
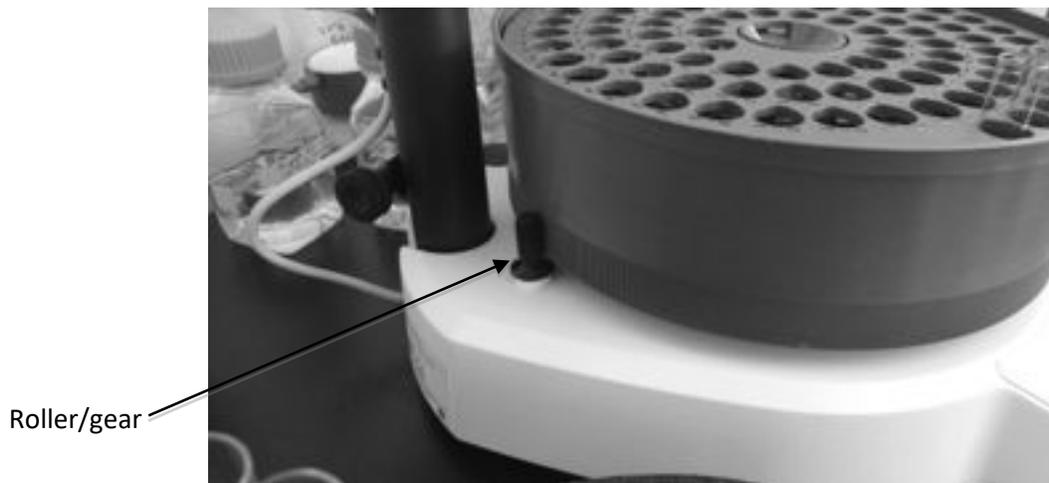


Fig. 5. Fraction collector carousel rubber advancement roller/gear.



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Fig. 6. *Release of roller to allow free rotation of the carousel.*

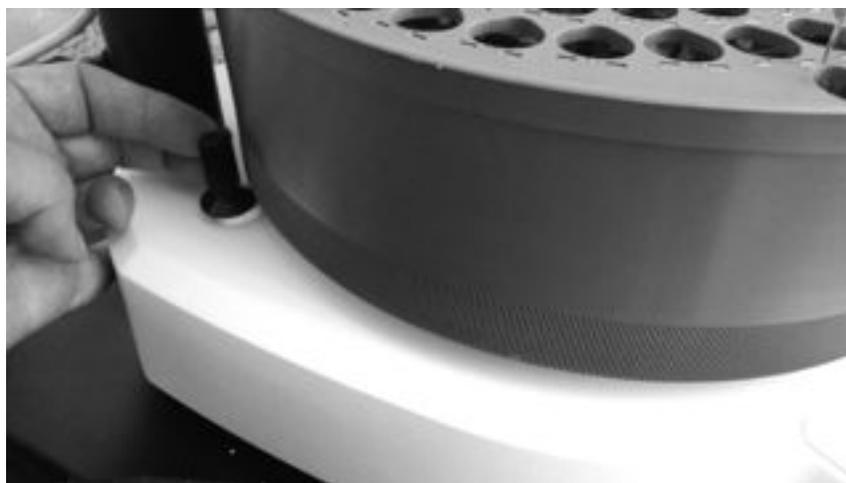


Fig. 7. *Location of tube #1 under the fraction collector drip outlet.*



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11. History

<i>Revision Number</i>	<i>Effective Date</i>	<i>Preparer</i>	<i>Description of Change</i>
0	16JUL15	David Frank	Initial release
1	21APR16	David Frank	Minor changes
2	10JAN20	Hetal Doshi	Added priming of the pump rinsing system, priming of inlets and purging of pump heads

AKTA pure 25 New Owner's User Guide

The exercise below will give a quick demonstration of how easy and intuitive the AKTA pure 25 will be for you in demonstrating downstream processing to your students.

Steps include:

- Opening the Unicorn software windows for manual control and programmable features
- Purging/priming pumps and tubing
- Loading a sample loop prior to injection on the column
- Editing a method
- Running a method

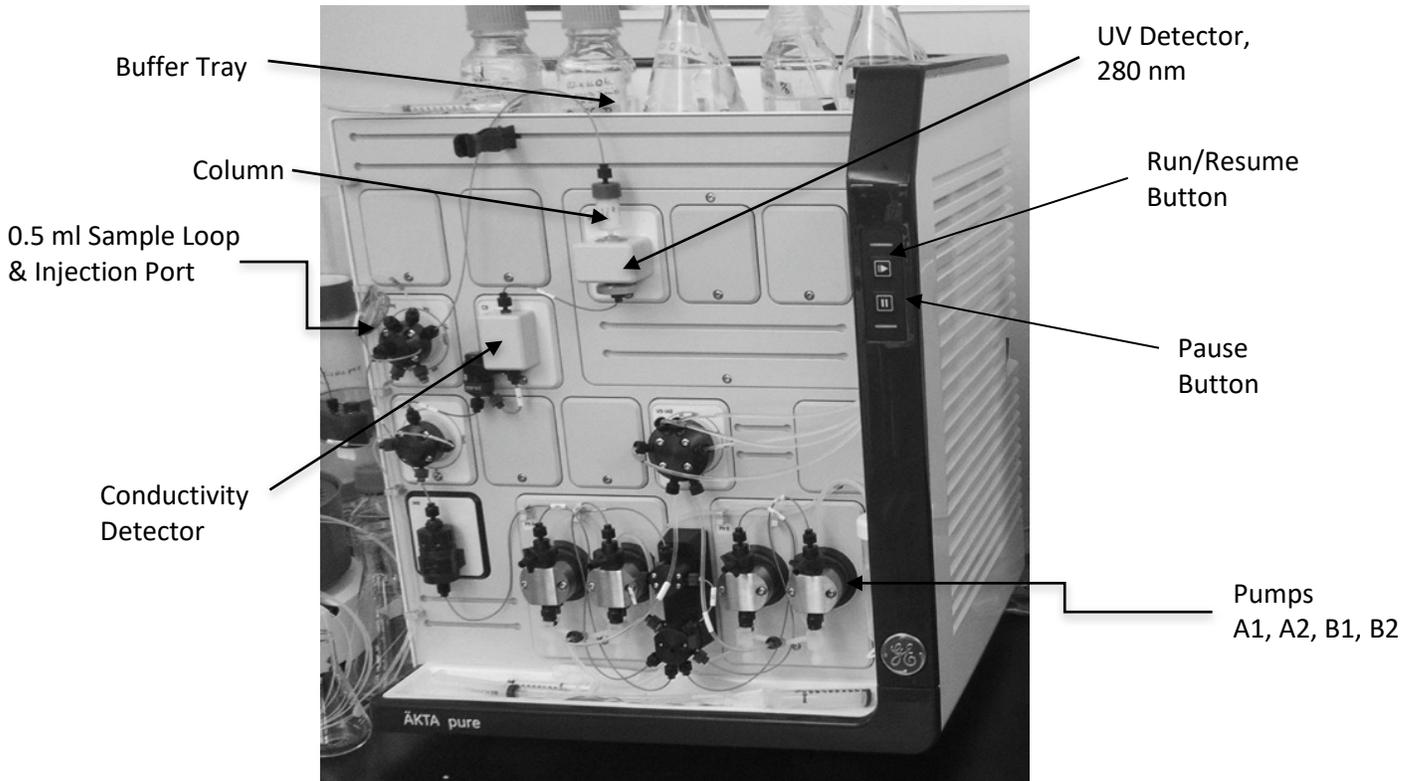
For this intro, a length of (restricted flow) tubing will be used in place of the column. You will also need filtered degassed deionized water or buffers, and a solution of 0.1% acetone in water (the sample).

Definitions:

Manual Load – position of the injection valve that allows the user to load the sample loop with sample by depressing the plunger of inserted syringe full of sample (commonly referred to as injecting, but in this context, injection has a different meaning; see below).

Injection – position of the injection valve that puts the sample loop in the flow path of mobile phase between the pumps and the column, such that the loaded sample flows from the sample loop onto/through the column and downstream path. The amount of sample that is injected onto the column can be specified in the method by editing prior to starting a chromatographic procedure.

Take a moment to identify the labeled components of the system:



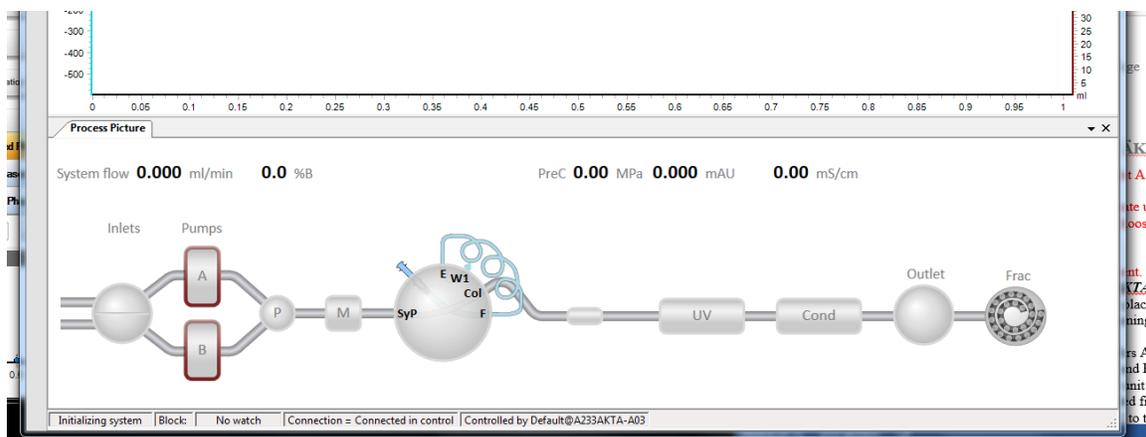
What to do with your new AKTA pure 25.

Great news – you have a brand new AKTApure automated chromatography instrument in your biomanufacturing lab!! Here is a brief introduction that will get you comfortable using most of the important functions of the instrument and allow you to successfully separate proteins in short order.

First the most important rule: never let air into the flow path. Running the pumps while dry will almost certainly damage them. Air space in the column can adversely affect resolution. Air in the us detector may result in uninterpretable A280 traces on the chromatogram. As with any liquid chromatography system air or gas in the system is the enemy, so make sure each of the inlet tubes (which deliver buffer to each of the pumps) is securely resting on the bottom of a buffer container (flask, bottle, etc).

1. Power up. Now you're ready to turn on the instrument (switch is on the right side, toward the rear), and the computer, if it's not already on.

2. Boot up Unicorn and get to know the manual System Controls. Open the Unicorn software windows: System Control, Method Editor, Evaluation, and Administration. Bring the System Control window to the forefront. If it is blank, go to the menu **System** and select **System Connect**. Place a check by the name of the instrument and click OK. You should now see the schematic of the flow path across the bottom of the screen, as shown here:



Now, if you click on each component you will see functions or parameters that you can change manually (via the software). For example, clicking the Inlets depicted (on the left side) allows you to switch the A inlet line and pumps between A1 and A2. Similarly you can switch between B1 and B2.

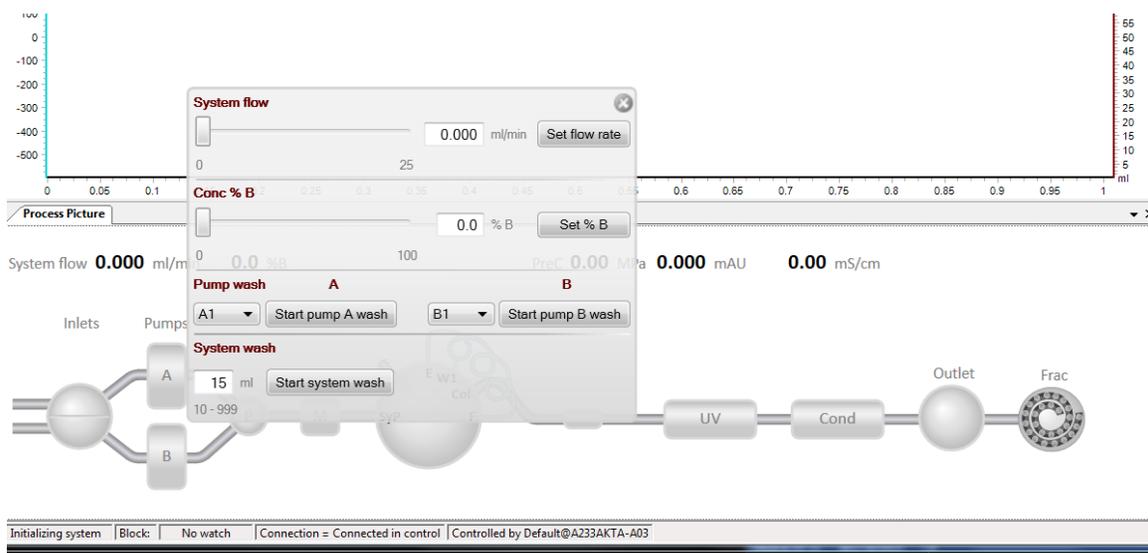
If the system is equipped with a pH detector, it will be shown between the Cond (conductivity) and Outlet positions. Click it and select the option to take it out of the flow path.



Starting with B1, attach a 10 ml syringe to the pump priming fitting and open the valve one full turn or more until you are easily able to withdraw 10 ml of liquid, which will insure the line is full of liquid and any bubbles are out of the line. Repeat these steps to prime every pump. Note that you will need to use the System Control window to manually switch A1 to A2 and

B1 to B2.

Clicking either of the depicted Pumps brings up a very useful menu that allows you to set the flow rate and buffer mix (expressed at %B), as well as quickly flushing the pumps and flow path up to the injection valve. Give it a try now. Use the mouse cursor to move the sliders then click the “Set” button to enter the new settings. *CAUTION [Changes take effect immediately upon clicking Set; if a column is installed and the flow rate is high enough, pressure will quickly rise, possibly damaging the column- so be careful here. This is less of an issue when running a programmed method, which will have pressure limits set to protect the column.]*



3. Wash the pumps. After confirming that you have enough mobile phase (i.e. buffer, water, cleaning solution or storage solution) in each reservoir, open the Pump manual control menu. Pumps should be washed in the order B2, B1, A2 and A1. Select **B2** from the dropdown menu in the **Pump wash** area of the dialog box, then choose the ‘**Wash Pump B**’ option. You will hear the injection valve reposition, sending liquid to waste, and the exhilarating sound of the pump working at high speed. This will quickly fill the pump and flow path up to the injection valve, then eluent will go to waste. The wash step

stops automatically after one minute. (This operation will not increase pressure on the column, if installed). Repeat with pump B1, A2 and A1.

What you have just done is the same procedure used to change buffers between operations, e.g. from storage in 20% EtOH to water to buffer for a chromatographic separation, or from chromatography buffer to CIP solutions for clean-in-place, CIP to water to EtOH for storage, etc).

4. Equilibrate the 'column' in start buffer. In the manual control panel for the pumps, move the slider to a flow rate of 1 ml/min and click '**Set flow rate**'. Pump A will begin to move the mobile phase at that rate through the injection valve, the 'column', detectors (uv, conductivity and/or pH) and on to the waste receptacle (or fraction collector if specified). After about 5 column volumes (CV), stop the flow, then end the 'run' by clicking the stop icon (a solid square) near the top of the window.

5. Load the Sample Loop. The default position for the Injection Valve is called 'Manual Load', in which the mobile phase flows from the pumps through the valve and then to the column and detectors. The sample loop is isolated from the flow path. It's good practice to rinse and fill the sample loop with start buffer prior to manually loading it with sample. To do so, fill a one ml syringe with start buffer (taking care to eliminate air bubbles) and attach it to the Luer lock fitting on the injection valve. Depress the plunger to force liquid into and through the loop (stopping before any air near the plunger can enter the sample loop). Excess liquid will go to the waste receptacle. Now repeat the process but fill the syringe with 'sample' (0.1% acetone), leaving the syringe attached to the fitting (to prevent siphoning of sample from the loop).

6. Select, Edit and Run a Method.

Select: In the Unicorn software, bring the Method Editor to the forefront. Under File, open a method of your choice, for example pretend to have installed a 1 ml ion exchange column; select a pre-programmed method for anion or cation exchange.

Edit: When the method opens, you will see each of the steps in the method (general settings, equilibration, sample injection, column wash, elution, re-equilibration and perhaps more). Clicking on any of the steps (called Phases) will result in the display of those parameters in the right pane. The Method Settings allows one to choose the appropriate column from the myriad available from GE, or to input custom column dimensions and characteristics. For this exercise, select a one ml ion exchange column. You will see the recommended settings for back pressure limit and flow rate. Here you may also indicate which pumps will be in use (A1, B1 are defaults), the measurement used in each setting (ml, min, or CV – column volumes) and which detectors are to be employed.

For each phase you will be able to designate the proper pump, % B in the eluent, override the flow rate, and set fraction collection details. Set the fraction collection ('Output') to waste for this demonstration. Changing the settings in one phase does not affect other phases, so you will need to set the output in each phase.

The elution phase will probably include a salt gradient; you may choose linear or step, and the initial and ending %B. Multiple step elution programs are also available.

After making the desired changes, choose Save As in the File menu, then rename the program and save it.

Run: Return to the System Control window. Under the File menu, choose Open, then double click the name of the method you just saved. The run will begin immediately, unless there is something amiss, e.g. the absence of tubes in the fraction collector, or the fraction collector is not in the ready position (which may not appear until the phase is initiated that calls for collection of fractions).

You can monitor the progress of the run in the System Control window. There should be an absorbance peak evident when the acetone sample passes through the uv detector.

7. Return to Storage. Upon completion, the system should be washed through with water, then 20% EtOH if long term storage (one week or longer) is desired. If you've run this demo with water, you can stop here, for short term storage. Repeat the initial pump wash step to change over to 20% EtOH, then run the pumps at 50%B for about 5 min at 5 ml/min. Make sure there is a large excess of 20% EtOH prior to starting.

8. Open the Evaluation window and the Results Navigator. The most recent chromatogram (yours) will be at the bottom of the list. Double click it to open. You can print it or choose to save it as a pdf.

For more information, please see the library of documentation on the GE Life Sciences website.

Quality Control Biochemistry: Downstream Processing

SOP: Monoclonal Antibody Aggregate Testing by HPLC SEC Analysis

Approvals:

Preparer: Dr. Matt Marshall	Date: 20MAR19
Reviewer: Robin Zuck	Date: 27MAR19
Reviewer: Dr. Maggie Bryans	Date: 04APR19
Reviewer: Hetal Doshi	Date: 28MAY19

1. Purpose:

1.1. To quantify the relative amount of aggregated and monomer molecules in solution of a monoclonal antibody (mAb) drug substance using size exclusion high performance liquid chromatography (HPLC) analysis.

2. **Scope:** Size Exclusion High performance liquid chromatography (HPLC) is an analytical chemistry technique for separating the components of a liquid sample based on molecular size. This SOP uses HPLC to detect the presence of monoclonal antibody aggregates or degradation products in a drug substance sample. HPLC is an FDA required test to adequately characterize the unconjugated mAb reagent of a drug product.

3. Summary of Method:

**Note: If the column is in long-term storage conditions, it requires ~3 hours of run-time to setup as described in 9.2. The system can be prepared for short-term storage conditions in mobile phase the previous day.*

- 3.1. Prepare the mobile phase solution
- 3.2. Power up the HPLC system and equilibrate with mobile phase solution for 60 minutes [if system is in short-term storage] or 120 minutes [if system is in long-term storage].
- 3.3. Prepare AdvanceBio SEC 300Å protein standards
- 3.4. Prepare mAb drug substance for HPLC analysis
- 3.5. Prepare buffer in which mAb drug substance is suspended.
- 3.6. Power up the HPLC system and equilibrate with mobile phase solution
- 3.7. Run an assay for using the prepared AdvanceBio SEC 300Å protein standards and mAb sample
- 3.8. Prepare HPLC system of short-term or long-term storage.
- 3.9. Compute the size of the conjugated and unconjugated mAb sample

4. References:

- 4.1. SOP: Buck Scientific BLC-20P HPLC Operation, document QCB 7, revision 0
- 4.2. SOP: Degassing a Solution by Helium Sparge, document number QCB 6, revision 0
- 4.3. User Guide for Agilent AdvanceBio SEC Columns

5. Definitions:

CV Column Volume; the volume (ml) of the column containing the stationary phase; CV= 2.91 ml for a standard size (4.6 X 250 mm) column

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<i>Equilibrium</i>	Running the mobile phase solution through the column prior to injecting the sample in order to bring the system into equilibrium
<i>Flow rate</i>	The rate (ml/min) that solution is pumped through the column. The operating flow rate is determined by the assay protocol.
<i>Helium Sparge</i>	Using a stream of helium bubbles to sweep dissolved air out of liquids (helium is virtually insoluble in most HPLC solvent solutions, so very little helium replaces the air)
<i>HPLC</i>	High Performance Liquid Chromatography
<i>Isocratic</i>	The composition of the mobile phase solution is constant; the system has only one pump.
<i>Long-term storage conditions</i>	In long-term storage the system is stored in 20% ethanol for one week or longer.
<i>Mobile phase</i>	The solvent solution used to carry the sample through the column
<i>PeakSimple</i>	Software used to collect and display data
<i>PSI</i>	Pounds per Square Inch
<i>Short-term storage conditions</i>	In short-term storage the system is stored in mobile phase solution for less than one week
<i>Size exclusion chromatography</i>	Separation based on molecule size. Molecules are separated on the basis of their exclusion from pores in the column packing material.
<i>Stationary phase</i>	The chromatography matrix through which the sample travels.

6. Precautions:

- 6.1. HPLC systems operate at high pressures. Personnel injury and equipment damage can result if maximum pressure is exceeded or the pump runs dry. Monitor pressure readings and solution level whenever the pump is running. If pressure exceeds 2500 psi or if the solution runs out, stop the pump immediately by pressing the RUN/STOP button. Do not set the flow rate higher than 1.5 ml/min with a 250 mm column.
- 6.2. Flow rate consistency is affected by the quality of the solutions. Use HPLC-grade solvents and filter solutions using a sub-micron filter (preferably 0.22 μm). Degas solutions prior to use.
- 6.3. To avoid microbial growth, do not leave the system in a high aqueous solution for a prolonged period. The system should be washed with a storage solution of 50% Methanol/H₂O or 50% Acetonitrile/H₂O if it is to be idle more than a few hours.
- 6.4. Methanol is flammable. Can cause blindness if swallowed. Vapor is harmful. Irritating to skin and eyes.

7. Responsibilities

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

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

8. Equipment and Materials:

- 8.1. Buck Scientific BLC-20P HPLC system pre-configured with:
 - 8.1.1. UV-Vis detector
 - 8.1.2. PeakSimple Chromatography Data System
 - 8.1.3. Computer system with PeakSimple software installed
 - 8.1.4. AdvanceBIO 300Å- 4.6mm x 150mm HPLC column. Product # PL1580-3301
- 8.2. HPLC-grade methanol
- 8.3. HPLC-grade water
- 8.4. monosodium phosphate monohydrate
- 8.5. disodium phosphate, heptahydrate
- 8.6. 20% Ethanol
- 8.7. AdvanceBio SEC 300Å protein standards. Product # 5190-9417
- 8.8. Post protein A chromatography monoclonal antibody (mAb) sample to be analyzed
- 8.9. Sample overflow waste beaker
- 8.10. Analytic balance
- 8.11. 500ml graduated cylinder
- 8.12. 500ml volumetric flask
- 8.13. 100ml volumetric flask
- 8.14. 1.5ml Eppendorf Tube
- 8.15. Stirring plate
- 8.16. 2- 500ml laboratory bottles (for mobile phase solution and waste)
- 8.17. 125ml laboratory bottle (for mobile phase to rinse the sample syringe)
- 8.18. Nalgene Rapid-flow filtration unit
- 8.19. 0.22µm syringe filters
- 8.20. 5 ml Luer-Lok syringe
- 8.21. 100uL HPLC sample syringe (Hamilton syringe)
- 8.22. Parafilm
- 8.23. Timer

9. Procedure:

- 9.1. Prepare 500ml of mobile phase solution (150mM Sodium Phosphate, pH 7.0).
 - 9.1.1. In a 500ml volumetric flask, add 3.976g monosodium phosphate, monohydrate and 12.379g disodium phosphate, heptahydrate and 480ml of MilliQ water.
 - 9.1.2. Add magnetic stirring bar, and stir until completely dissolved.
 - 9.1.3. Check the pH.
 - 9.1.4. If required, adjust pH to 7.0±0.1 with 1N phosphoric acid. Bring the volume to 500ml.
 - 9.1.5. Filter the mobile phase solution using a Nalgene Rapid-flow filtration unit into an autoclaved 1L laboratory bottle.

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- 9.1.6. Degas the mobile phase solution per the Degassing a Solution by Helium Sparge SOP QCB 6.
 - 9.1.6.1. Transfer approximately 10ml of mobile phase solution into a labeled 125ml laboratory bottle. This will be used for rinsing the sample syringe.
 - 9.1.6.2. Label an empty 500ml laboratory bottle as mobile phase solution waste and place waste line into it, cover with Parafilm.
- 9.2. A) System in short-term storage: Power up the HPLC system and turn on the UV-Vis detector, equilibrate with mobile phase solution at a flow rate of 0.25mL/min for 30 minutes.
 - B) System in long-term storage: Power up the HPLC system and turn on the UV-Vis detector, flush column with 5 column volumes of HPLC-grade water at a flow rate of 0.25mL/min for 60 minutes, then 10 column volumes mobile phase solution at a flow rate of 0.25mL/min for 120 minutes.
- 9.2.1. Power up the HPLC system components and start the PeakSimple data collection software [Refer to SOP: Buck Scientific BLC-20P HPLC Operation, document QCB 7, revision 0]
 - 9.2.1.1. Switch the system to mobile phase solution.
 - 9.2.1.1.1. Verify that the pump is off. The Run LED should be off
 - 9.2.1.1.2. Place the intake line into the mobile phase solution bottle and cover with Parafilm
 - 9.2.1.1.3. Verify that the frit is submerged in the solution.
 - 9.2.1.1.4. Place the outlet line into the mobile phase waste bottle.
 - 9.2.1.1.5. Place the sample overflow line into a small waste beaker
 - 9.2.1.2. Purge the intake line and prime the pump
 - 9.2.1.2.1. Attach an empty 5ml Luer-Lok syringe to the purge valve
 - 9.2.1.2.2. Open the prime/purge valve by turning it two full turns counter-clockwise.
 - 9.2.1.2.3. Watching the intake line for bubbles, slowly draw the syringe plunger until it is fully drawn. Mobile phase solution and bubbles should fill the syringe.
 - 9.2.1.2.4. Close the prime /purge valve by rotating it clockwise until it stops.
 - 9.2.1.2.5. Remove the syringe from the purge valve and expel the contents into the waste bottle.
 - 9.2.1.2.6. Repeat attaching the syringe to the purge valve, drawing bubbles and solution into the syringe, and expelling into the waste bottle until free of bubbles (generally 10-15 ml of mobile phase are needed).
 - 9.2.1.3. Start the pump and gradually increase the flow rate to 0.25ml/min over 5min.
 - 9.2.1.3.1. Set the initial flow rate to 0.1 ml/min:
 - 9.2.1.3.1.1. Press the MODE button on the front panel repeatedly until the Flow LED turns on. The current flow rate (in ml/min) appears on the digital display.
 - 9.2.1.3.1.2. Press the Flow up arrow button to increase the flow rate setting and press the down arrow button to decrease the flow rate setting.
 - 9.2.1.3.1.3. Repeat pressing the Flow arrow buttons until 0.10 is displayed.
 - 9.2.1.3.1.4. Start the pump by pressing the RUN/STOP button. The LED should turn on.

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- 9.2.1.3.2. Monitor the pressure reading and mobile phase level.
 - 9.2.1.3.2.1. Display the pressure reading by pressing the MODE button repeatedly until the Pressure LED turns on. The current pressure (in psi) appears on the digital display.
 - 9.2.1.3.2.2. Verify that the waste is dripping into the waste bottle.
 - 9.2.1.3.2.3. If the pressure exceeds 2500 psi or the mobile phase runs low stop the pump immediately by pressing the RUN/STOP button.
 - 9.2.1.3.2.4. Gradually increase the flow rate to 0.25 ml/min over 5 minutes.
 - 9.2.1.3.2.4.1. Increase the flow rate in 0.1 ml/min increments using the Flow up arrow button.
 - 9.2.1.3.2.4.2. Monitor the pressure readings until a flow rate of 0.25ml/min is achieved and the pressure reading is stable.
- 9.2.2. Ensure the UV-Vis detector has been running for at least 15 minutes before proceeding. Set the UV-Vis detector wavelength to 220 nm and autozero the detector.
 - 9.2.2.1. Set the UV-Vis detector wavelength by pressing the λ up and down buttons until 220nm is displayed.
 - 9.2.2.2. Press the Autozero button in the front panel.
- 9.3. While system is equilibrating, reconstitute AdvanceBio SEC 300Å protein standards [Concentration=3.8mg/ml].
 - 9.3.1. Reconstitute lyophilized mixture vial contains 3.8mg total protein.
 - 9.3.2. Add 1,000µL of freshly prepared mobile phase solution (150mM Sodium Phosphate, pH 7.0).
 - 9.3.3. Invert the vial several times gently (do not vortex).
 - 9.3.4. Place in a sonicating water bath. Pulse for several minutes in 30s intervals with 15s breaks to fully reconstitute the sample; avoid elevating temperature.
 - 9.3.5. Manufacture recommendations are to prepare aliquots of this standard stock solution and store at 2-8°C for up to 1 year. However, this may result in degradation of the 1 KD protein standard. This can be avoided by storing single-use aliquots at -20°C.
- 9.4. Prepare a 0.152mg/ml working stock of AdvanceBio SEC 300Å protein standards
 - 9.4.1. To produce protein standards at [0.152mg/ml], place 40µL of reconstituted AdvanceBio SEC 300Å protein standards [Concentration=3.8mg/ml] into labeled 1.5ml Eppendorf tube.
 - 9.4.2. Add 960µL of freshly prepared mobile phase solution, 150mM Sodium Phosphate, pH 7.0.
 - 9.4.3. Invert the vial several times gently (do not vortex).
 - 9.4.4. Gently pipette up and down several times to mix.
 - 9.4.5. Transfer with pipette and filter with 0.22µm syringe filter into a sterile 1.5ml Eppendorf tube
 - 9.4.6. Label as 'S' for AdvanceBio SEC 300Å protein standards
- 9.5. Prepare monoclonal antibody (mAb) sample for HPLC analysis.

**Note: Post protein A chromatography mAb sample contains 0.083M Sodium Citrate pH3 and 0.167M Tris pH 9.*

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- 9.5.1. The mAb is diluted in freshly prepared mobile phase solution (150mM Sodium Phosphate, pH 7.0) to a final concentration between 0.024 - 0.096mg/ml.
- 9.5.2. For a sample with 1.2mg/ml, place 40 μ L of post protein A chromatography mAb drug substance into labeled 1.5ml Eppendorf tube.
- 9.5.3. Add 960 μ L of freshly prepared mobile phase solvent containing 150mM Sodium Phosphate, pH 7.0.
- 9.5.4. Invert the vial several times gently (do not vortex).
- 9.5.5. Gently pipette up and down several times to mix.
- 9.5.6. Transfer with pipette and filter with 0.22 μ m syringe filter into a sterile 1.5ml Eppendorf tube
- 9.5.7. Label as 'M' for mAb sample
- 9.6. Prepare monoclonal antibody (mAb) buffer [1,000 μ l 0.1M Sodium Citrate, pH 3 + 200 μ l 1M Tris pH 9] for HPLC analysis.
 - 9.6.1. Dilute buffer that post protein A chromatography mAb is suspended in using the same dilution as used in step 9.5.
 - 9.6.2. Invert the vial several times gently (do not vortex).
 - 9.6.3. Gently pipette up and down several times to mix.
 - 9.6.4. Transfer with pipette and filter with 0.22 μ m syringe filter into a sterile 1.5ml Eppendorf tube.
 - 9.6.5. Label as 'B' for buffer.
- 9.7. The UV-Vis detector needs to warm up for 60 minutes prior to collecting data, monitor the UV-Vis signal for stability. For each sample (S, M, B) run an assay for 10 minutes at 0.25ml/min per the HPLC Operation SOP. See 10.1-10.3 for example chromatograms of S, M & B.
 - 9.7.1. Use PeakSimple to start a new 10 minute run.
 - 9.7.2. Autozero the UV-Vis detector.
 - 9.7.3. Setup autosampler to record data for 10 minutes.
 - 9.7.3.1. In PeakSimple software select EDIT – Channels.
 - 9.7.3.2. Under Channel 1, select Details.
 - 9.7.3.3. Change default display limits to 75mV MAX, -10mV MIN.
 - 9.7.3.4. Change end time to 10 mins.
 - 9.7.3.5. Leave other settings as default and click OK.
 - 9.7.3.6. In PeakSimple software select EDIT – Overall
 - 9.7.3.7. Change default display period Start: 1 min., End: 10 min.
 - 9.7.3.8. Leave other settings as default and click OK.
 - 9.7.4. Load and inject sample.
 - 9.7.4.1. Rinse syringe 3x with fresh mobile phase solution.
 - 9.7.4.2. Fill Hamilton syringe with 100 μ l of sample.
 - 9.7.4.3. Ensure the sample is free of air bubbles
 - 9.7.4.4. Insert the syringe into sample injection port.
 - 9.7.4.5. Inject sample into HPLC machine, ensuring air bubbles do not enter machine
 - 9.7.4.6. Twist sample port injection port switch from LOAD position to INJECT position
 - 9.7.4.7. Wait 10 seconds

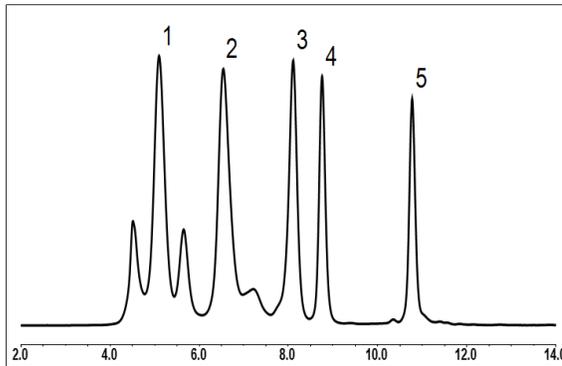
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- 9.7.4.8. Twist sample port back to LOAD position.
- 9.7.4.9. Remove syringe and empty contents into waste beaker.
- 9.7.4.10. Rinse syringe 3x with fresh mobile phase solution.
- 9.7.4.11. To run additional samples, repeat steps 9.7.4.2 through 9.7.4.10.
- 9.7.4.12. After the last sample has run, rinse syringe 3x with MilliQ water and 3x with 20% ethanol.
- 9.7.5. Note the time at the center of the sample peak on the chromatograph.
 - * The AdvanceBio SEC 300Å protein standards will contain 5 distinct peaks
- 9.7.6. Save the data to a separate chromatogram file.
- 9.7.7. View the results [VIEW – Results..] and copy the data to a separate sheet in an Excel workbook.
- 9.7.8. Save chromatogram file with new name, and print chromatogram.
- 9.7.9. Retention time and area associated with each peak will print with the chromatogram.
- 9.8. Prepare HPLC system for short-term (1 week or less) or long-term (longer than 1 week) storage.
 - 9.8.1. Short-term storage (1 week or less): Wash the system with mobile phase solution for 30 minutes at a flow rate of 0.25ml/min.
 - 9.8.2. Long-term storage (longer than 1 week):
 - 9.8.2.1. Flush the column with a minimum of 10 column volumes with 0.2µm filtered and degassed MilliQ water to remove buffer salts.
 - 9.8.2.2. Flush the column with a minimum of 10 column volumes with 0.2µm filtered and degassed 20% Ethanol.
- 9.9. Graph the calibration curve using excel:
 - 9.9.1.1. Add new sheet to Excel workbook with 2 columns. Label the left column “Retention Time (min)” and the right column “Protein Size (kDa)”
 - 9.9.1.2. Fill in the Protein Size column with the values 670, 150, 45, 17, 1.
 - 9.9.1.3. Fill in the Retention Time column with the retention time of each peak corresponding to the AdvanceBio SEC 300Å protein standards.
 - 9.9.1.4. Highlight the data table and create a scatter chart.
 - 9.9.1.4.1. Insert Tab > Scatter Chart.
 - 9.9.1.5. Reformat the y-axis (Protein Size) to the log scale.
 - 9.9.1.5.1. Right click on y-axis > Format Axis. Axis Options Tab > Check Logarithmic scale [Base 10]
 - 9.9.1.6. Insert a Trendline.
 - 9.9.1.6.1. Click on chart > + sign in upper right > Trendline ► More Options...> Trendline Options Tab > Select ‘Exponential’, Select ‘Display Equation on Chart’, Select ‘Display R-squared value on chart’
- 9.9.2. Estimate size of mAb drug product by inserting retention time into equation of line derived from AdvanceBio SEC 300Å protein standards
 - 9.9.2.1. Substitute ‘x’ in equation for retention time of mAb drug product peak.
 - 9.9.2.2. Solve for ‘y’, protein size in kDa.

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10. Attachments:

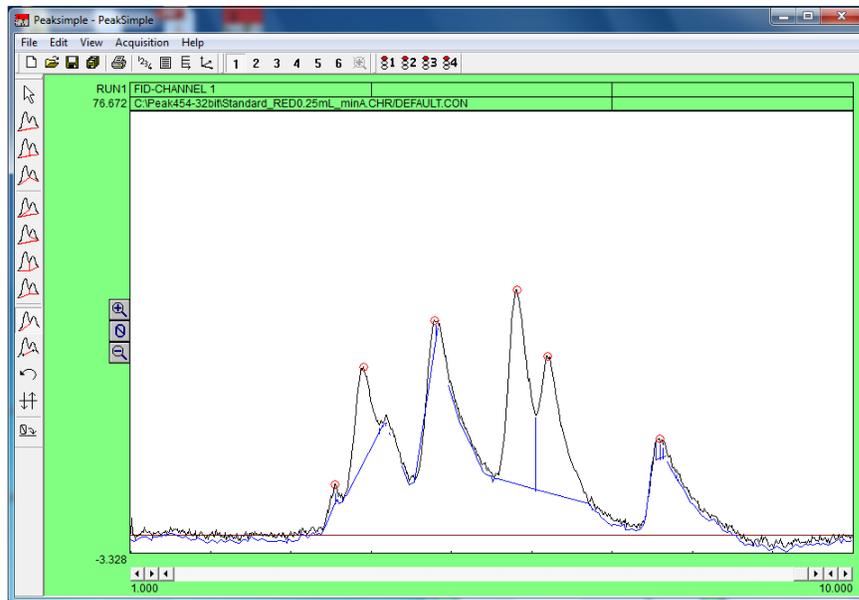
10.1. Protein standard 5190-9417 separated on an Agilent AdvanceBio SEC 300Å Column, 4.6 x 300 mm (PL1580-5301).



Peak #	Analyte	MW (daltons)
1	Thyroglobulin (bovine)	670,000
2	γ-globulin (bovine)	150,000
3	Ovalbumin (chicken)	45,000
4	Myoglobin (equine heart)	17,000
5	Angiotensin II (human)	1,000

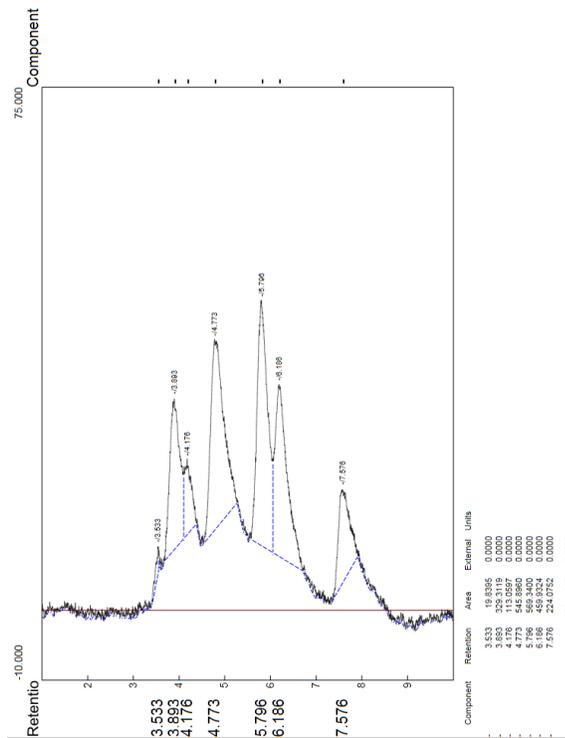
*Retention times will vary with different HPLC conditions, column and instrument.

10.2. Chromatogram of AdvanceBio SEC 300Å protein standards run on AdvanceBIO 300Å-4.6mm x 150mm HPLC column (Product # PL1580-3301) at a rate of 0.25ml/min. x-axis: Retention time; y-axis: absorbance at 220nm as viewed in PeakSimple software.

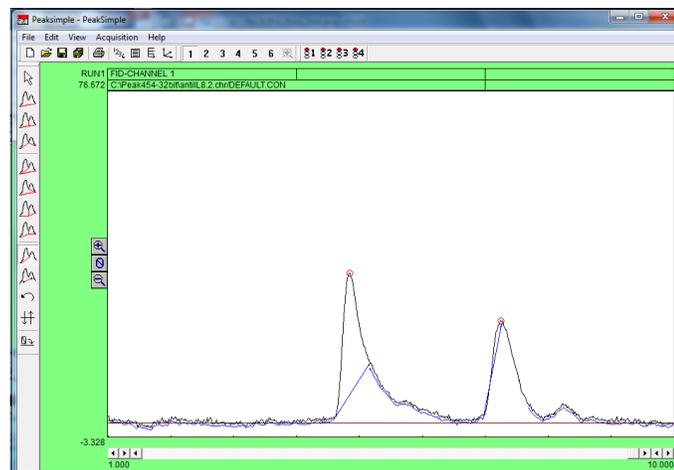


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10.3. Chromatogram of AdvanceBio SEC 300Å protein standards run on AdvanceBIO 300Å-4.6mm x 150mm HPLC column (Product # PL1580-3301) at a rate of 0.25ml/min. x-axis: Retention time; y-axis: absorbance at 220nm as viewed on a Peaksimple print-out.

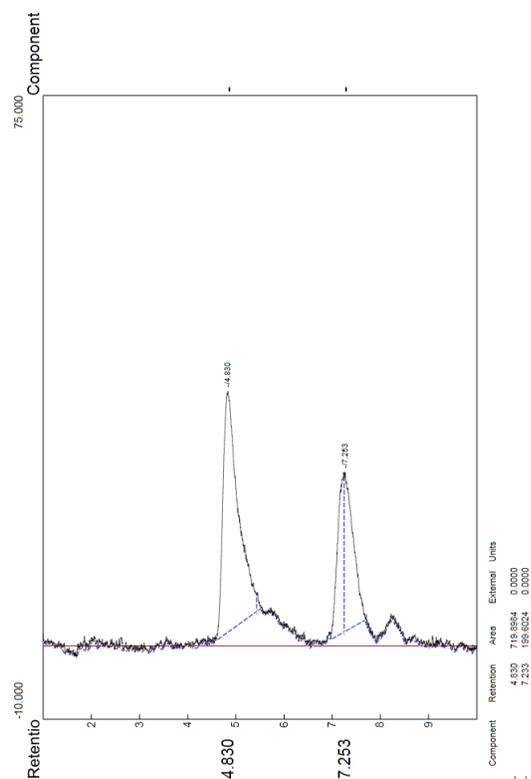


10.4. Chromatogram of anti-IL-8 mAb drug product run on AdvanceBIO 300Å- 4.6mm x 150mm HPLC column (Product # PL1580-3301) at a rate of 0.25ml/min x-axis: Retention time; y-axis: absorbance at 220nm as viewed in Peaksimple software.

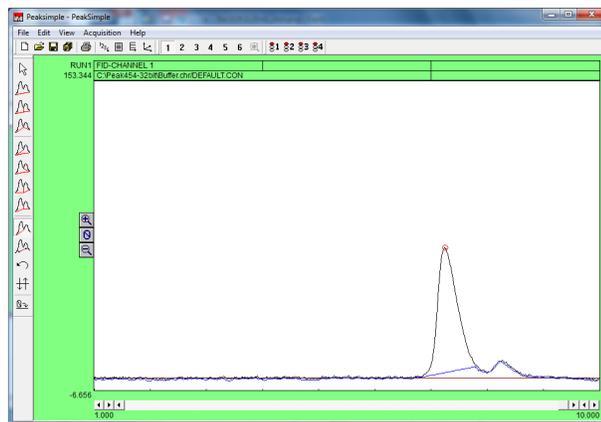


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- 10.3. Chromatogram of anti-IL-8 mAb drug product run on AdvanceBIO 300Å- 4.6mm x 150mm HPLC column (Product # PL1580-3301) at a rate of 0.25ml/min
x-axis: Retention time; y-axis: absorbance at 220nm as viewed on a Peaksimple print-out.

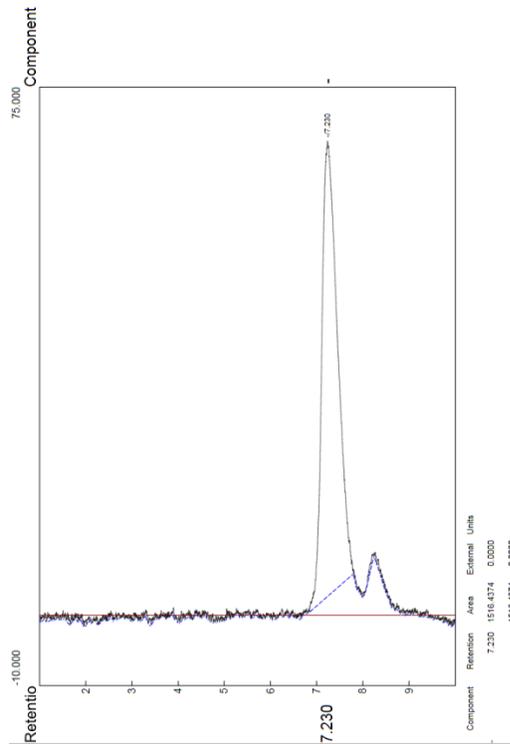


- 10.4. Chromatogram of mAb buffer run on AdvanceBIO 300Å- 4.6mm x 150mm HPLC column (Product # PL1580-3301) at a rate of 0.25ml/min. x-axis: Retention time; y-axis: absorbance at 220nm as viewed in Peaksimple software.

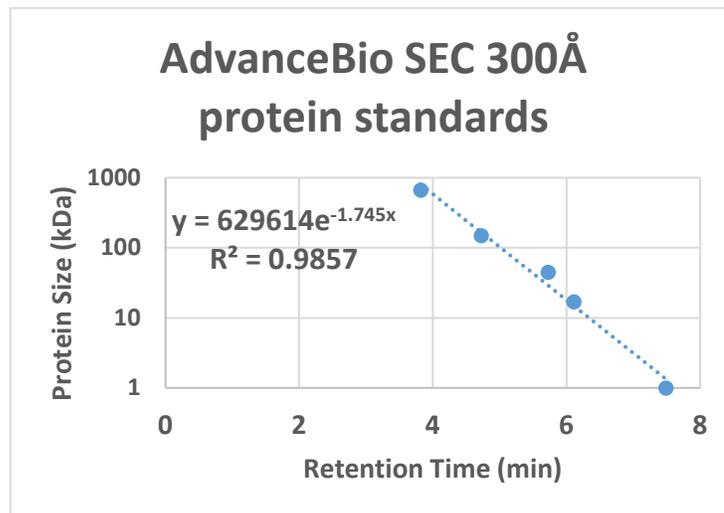


SOP: Monoclonal Antibody Aggregate Testing by HPLC SEC Analysis

10.5. Chromatogram of mAb buffer run on AdvanceBIO 300Å- 4.6mm x 150mm HPLC column (Product # PL1580-3301) at a rate of 0.25ml/min. x-axis: Retention time; y-axis: absorbance at 220nm as viewed in Peaksimple software.



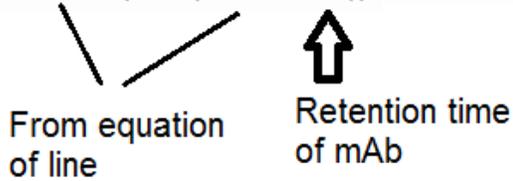
10.4 Standard curve of AdvanceBio SEC 300Å protein standards (as presented above in 10.2 & 10.3)



SOP: Monoclonal Antibody Aggregate Testing by HPLC SEC Analysis

10.5 Example of equation typed into Excel to solve for the size of a mAb that exhibited a retention time of 4.79 seconds using the equation of the exponential line shown in 10.4.

=629614*(EXP(-1.745*4.79)) =147.571 KiloDaltons



11. History:

Name	Date	Amendment
Dr. Matt Marshall	04APR2019	Initial release

SOP: SDS-PAGE Protein Gel Electrophoresis

Approvals:

Preparer: Robin M. Zuck

Date: 15APR19

Reviewer: Hetal Doshi

Date: 18APR19

Reviewer: Dr .Maggie Bryans

Date: 20APR19

1. Purpose:

1.1. To describe the steps necessary to perform an SDS-PAGE analysis of a protein sample

2. **Scope:** This SOP covers the preparation of SDS-PAGE protein gels suitable for Coomassie staining or Western Blotting.

3. Responsibilities:

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

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

4. References:

4.1. XCell SureLock® Mini-Cell User Guide, Publication Part number IM-9003

4.2. GelCode Blue Stain Reagent Instructions, Thermo Scientific

5. Precautions:

5.1. Routine care should be exercised in the handling of buffers and samples of biological materials, which may have harmful biological activity in the case of accidental ingestion, needle stick etc.

5.2. Gloves, a lab coat and protective eyewear should be worn when handling buffers and samples.

5.3. Always wear gloves when handling polyacrylamide gels.

6. Materials:

6.1. 4-20% Tris-Glycine Gel, Invitrogen Novex WedgeWell (Reference # XP04200BOX)

6.2. 10X Tris/Glycine/SDS Running Buffer, BIO-RAD Catalog # 161-0732

6.3. GelCode Blue Stain Reagent, Thermo Scientific (Product # 24590)

6.4. XCell SureLock Gel Box

6.5. Gel Knife

6.6. Power Supply

6.7. Heating block set at 95°C

6.8. 1.5 ml microfuge tubes

6.9. 2X Laemmli Sample buffer, BIO-RAD catalog #161-0737 with added β mercaptoethanol

6.10. Precision Plus Protein Kaleidoscope Ladder, (BioRad catalog # 161-0375)

6.11. Gel loading pipette tips

6.12. 10 ml syringe and 21G2 needle

6.13. Ice bucket and ice

7. Procedure:

7.1. Prepare Samples.

Note: protein samples should be kept on ice while preparing the samples.

7.1.1. Fill the needed number of holes in the heating block with Milli Q water.

7.1.2. Turn the heat block on and set the temperature to 95°C to preheat.

SOP: SDS-PAGE Protein Gel Electrophoresis

- 7.1.3. Label one microfuge tube for each sample.
 - 7.1.4. For each sample to be analyzed;
 - 7.1.4.1. Determine the amount of total protein to be loaded on the gel.
 - 7.1.4.2. Using the sample total protein concentration, calculate the sample volume equal to the amount of protein to be loaded.
 - 7.1.4.3. Calculate the volume of Milli Q water needed to add to the sample volume to bring the combined water + sample volume to 15 μ l.
 - 7.1.4.4. Add 15 μ l of 2X to this sample tube.
 - 7.1.4.5. If the sample protein concentration is too low to add the desired protein amount in 15 μ l, it is possible to load 40 μ l in a well. Prepare the sample using a sample volume of up to 20 μ l. If the sample volume needed is less than 20 μ l add enough Milli Q water to the tube to bring the volume to 20 μ l. Then add 20 μ l of 2X sample buffer to this tube. For these samples 40 μ l will be loaded into the well of the gel.
 - 7.1.4.6. Combine the calculated sample, water and 2X sample buffer volumes for each sample in the labeled sample microfuge tube.
 - 7.1.5. Heat each of the prepared sample tubes at 95°C in the heating block for 2 minutes.
 - 7.1.6. Return the sample tubes to ice until they are loaded on the gel.
 - 7.1.7. Just prior to loading give the sample tubes a quick spin in a table top centrifuge to collect the sample in the bottom of the tube.
- 7.2. Prepare 800ml of 1X Tris/Glycine/SDS Running Buffer.
 - 7.2.1. Add 80 ml of 10X Tris/Glycine/SDS Running Buffer to 720 ml of MilliQ water
 - 7.2.2. Mix gently to avoid foaming.
 - 7.3. Prepare the gel/gels and assemble the gel box. See Attachment 8.1.
 - 7.3.1. Cut open the gel cassette pouch and remove the gel.
 - 7.3.2. Discard the gel packaging buffer.
 - 7.3.3. Rinse the gel cassette with Milli Q water.
 - 7.3.4. Remove the tape covering the slot on the back of the gel cassette.
 - 7.3.5. Carefully to avoid damaging the wells, remove the comb from the top of the gel cassette by sliding the comb straight out.
 - 7.3.6. Place the Buffer Core into the Lower Buffer Chamber
 - 7.3.7. Place the Gel Tension Wedge into the Gel Box behind the Buffer Core. Make sure the Gel Tension Wedge is in the unlocked position. The Gel Tension Wedge should rest on the bottom of the lower buffer chamber. (See attachment 8.2)
 - 7.3.8. Insert the gel cassette into the lower buffer chamber in front of the core with the shorter well side of the cassette facing the buffer core.
 - 7.3.9. If you are running two gels place the second gel cassette in the lower buffer chamber behind the buffer core with the shorter well side of the cassette against the buffer core.
 - 7.3.10. If you are running only one gel insert a Buffer Dam in place of the second, rear, gel cassette.
 - 7.3.11. Pull the Gel Tension Wedge Lever toward the front of the Gel Box until it comes to a firm stop. The gels or gel and buffer dam should now be held firmly against the buffer core.

SOP: SDS-PAGE Protein Gel Electrophoresis

- 7.3.12. Fill the upper buffer chamber with 200ml of the 1X running buffer, use enough buffer to completely cover the sample wells.
- 7.3.13. Make sure that the upper buffer chamber is not leaking. If the buffer level drops, reseal the gels by repeating steps 7.3.8 through 7.3.11.
- 7.3.14. Fill the lower chamber with the remaining 600 ml of running buffer.
- 7.3.15. Using the syringe and 21G2 needle carefully flush each of gel wells with 1X running buffer.
- 7.3.16. Using gel loading pipette tips, carefully load the sample into the bottom of the designated well being careful not to introduce bubbles in the well.
 - 7.3.16.1. Remember to load one well with 5 μ l of the Protein Ladder
 - 7.3.16.2. Load each sample well with the with the total prepared sample volume, (either 30 μ l or 40 μ l).
 - 7.3.16.3. Load any unused wells with 30 μ l of 1X sample buffer, (15 μ l of Milli Q water + 15 μ l 2X sample buffer).
- 7.3.17. Place the lid on the gel box and check that it is firmly seated.
- 7.4. With the power supply OFF, connect the electrodes from the gel box to the power supply as follows;
 - 7.4.1. Connect the positive, red jack to the red port.
 - 7.4.2. Connect the negative, black jack to the black port.
 - 7.4.3. Turn the power on,
 - 7.4.4. Set the power supply to constant voltage
 - 7.4.5. Using the up and down arrows adjust the voltage to 125 volts.
 - 7.4.6. The gel should run for 60 to 90 minutes. Monitor the progress of the dye front and turn the power supply OFF when the dye front is slightly above the gel foot. The colored bands of the ladder should be visible and separated.
 - 7.4.7. With the power OFF, disconnect the power supply from the gel box.
 - 7.4.8. Remove the lid from the box. Unlock the Gel Tension Wedge and remove the gel cassette.
- 7.5. Remove the gel.
 - 7.5.1. Prepare a container for washing and staining the gel. It should be large enough to hold the gel with a volume of about 50ml. Place about 20 ml of Milli Q water in this container
 - 7.5.2. Lay the gel cassette on the bench well side up. Insert the gel knife at the bottom corner of the cassette between the 2 plastic plates being careful not to contact the gel. Angle the knife up and down to separate the plates. You will hear a cracking sound. Place the knife in the opposite bottom corner and separate the plates on that side.
 - 7.5.3. Carefully open the cassette, the gel will be attached to one of the plates. Discard the plate that is not holding the gel.

IF THE GEL IS TO BE USED FOR A WESTERN BLOT CONTINUE USING THE SOP FOR THE APPROPRIATE WESTERN BLOTTING PROCEDURE.

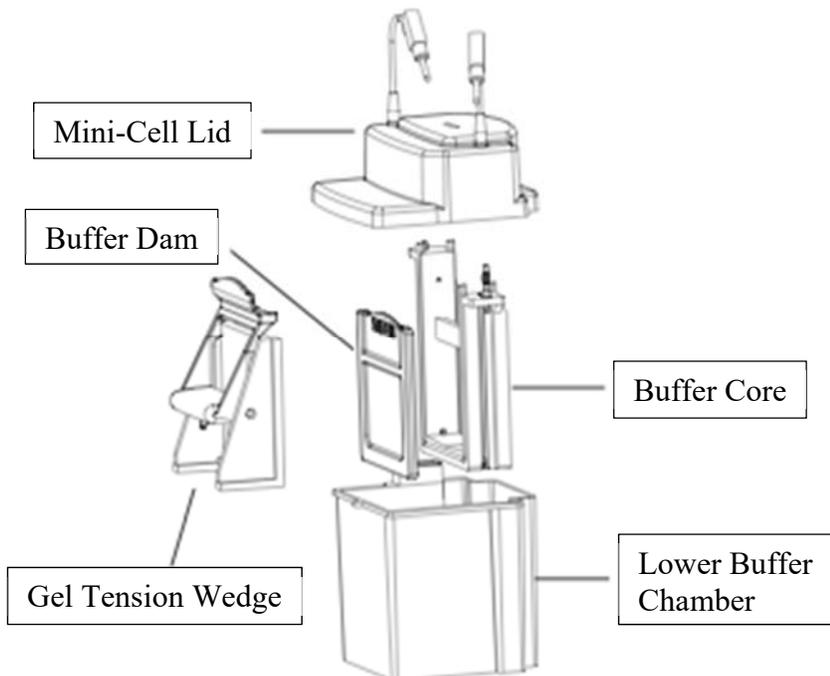
SOP: SDS-PAGE Protein Gel Electrophoresis

- 7.5.4. If the gel is attached to the shorter plate use the gel knife use the knife to gently lift a bottom corner of the gel. Using both hands to lift the gel transfer the gel to the prepared container with Milli Q.
- 7.5.5. If the gel is attached to the longer slotted plate, carefully use the knife to push the gel foot up through the slot so that the gel can be removed from the plate. Using both hands gently lift the gel and transfer the gel to the prepared container containing Milli Q water.
- 7.5.6. Allow the gel to wash in the for 5 minutes on a rotary platform. Replace the Milli Q water with 20 ml of fresh Milli Q and repeat this 5 minute wash twice more for a total of three washes.
- 7.6. If a Western Blot is to be prepared using this SDS-PAGE gel refer to SOP: XX for preparing a Western Blot.
- 7.7. Stain the gel using Coomassie Blue stain
 - 7.7.1. Drain the water from the container holding the gel and add approximately 20 ml of GelCode Blue Stain. Cover the box with parafilm and place on a rotary plate. Incubate the gel with the stain for 6 hours to overnight.
- 7.8. Destain the gel
 - 7.8.1. Decant the stain from the gel and add approximately 20 ml of Milli Q water and return to the rotary plate for 20 minutes. Repeat this wash process two more times for a total of 3 washes.
- 7.9. The dyed gel can now be imaged and analyzed.

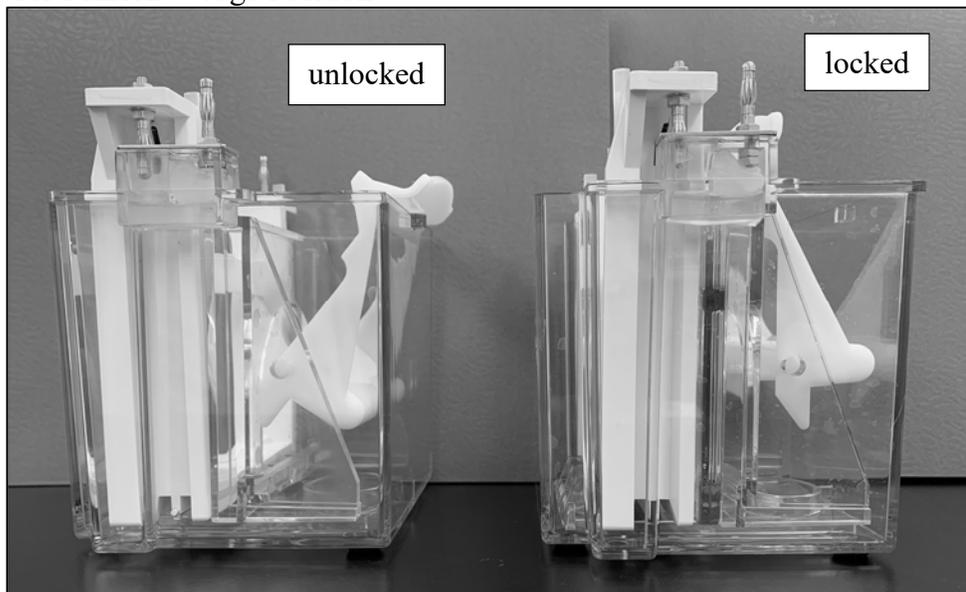
SOP: SDS-PAGE Protein Gel Electrophoresis

8. Attachments

8.1. Gel Box



8.2. Gel Tension Wedge Position



SOP: SDS-PAGE Protein Gel Electrophoresis

9. History:

Revision Number	Effective date	Preparer	Description of Change
0	20/04/19	Robin Zuck	Initial release

SOP: Degassing a Solution by Helium Sparge

Approvals

Preparer: John Buford
Reviewer: Jack O'Neill
Reviewer: Tim Kull
Reviewer: Dr. Margaret Bryans

Date: 24SEP13
Date: 24SEP13
Date: 25SEP13
Date: 26SEP13

1. Purpose

1.1. Remove dissolved atmospheric gases from a solution by means of sparging with helium.

2. Scope and Applicability

2.1. Applicable to preparing solvent solutions for use in High Performance Liquid Chromatography (HPLC). Solution volume is limited to the range of 25 mL to 800 mL.

3. Summary of Method

3.1. Helium gas is delivered from a compressed gas tank to a porous metal sparger that is placed in a bottle of solution. Helium is bubbled through the solution for 15 minutes.

4. References

4.1. Airgas® Operation and Safety Instructions For Specialty Gas Regulation Equipment, form #320-517 Rev. 5/03

5. Definitions

Helium sparge Using a stream of helium bubbles to sweep dissolved air out of liquids (helium is virtually insoluble in most HPLC solvent solutions, so very little helium replaces the air)

HPLC High Performance Liquid Chromatography

PSI Pounds per Square Inch

6. Precautions

- 6.1. A helium tank is compressed to pressures up to 2000 PSI. A sudden release of pressure can cause serious damage to personnel and equipment. Handle the helium tank and gas regulator with care and wear eye protection.
- 6.2. Helium sparging entails some risk of changing the composition of an HPLC solvent solution by selectively evaporating the more volatile components. Avoid an excessively vigorous flow of helium.

7. Responsibilities

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

SOP: Degassing a Solution by Helium Sparge

Equipment and Materials

- 7.3. Laboratory grade (99.9+%) helium in a compressed gas cylinder (helium tank)
- 7.4. Airgas® gas cylinder regulator pre-connected to the helium tank
- 7.5. Porous metal sparger
- 7.6. Flexible gas tubing
- 7.7. Laboratory bottle containing HPLC storage solution (e.g. 50% MeOH/H₂O or 50% Acetonitrile/H₂O).
- 7.8. Solution to be sparged (25 to 800 mL)
- 7.9. Empty laboratory bottle (50, 100, 250, 500, or 1000 mL)
- 7.10. Two-hole stopper that is sized to the empty laboratory bottle (size # 1 for 50 or 100 mL bottle, size #6.5 for 250, 500, or 1000 mL bottle)
- 7.11. Laboratory grade water in a wash bottle
- 7.12. Waste beaker
- 7.13. Timer

8. Procedure

- 8.1. Verify that all personnel in the area are wearing eye protection.
- 8.2. Connect the sparger to the helium tank if it is not connected already:
(See Figure 1 for location of valves.)
 - 8.2.1. Turn the gas pressure off by turning the regulator valve counter-clockwise for two full turns (in the direction marked “DECREASE”).
 - 8.2.2. Close the delivery valve by turning it clockwise until it stops.
 - 8.2.3. Close the tank valve by turning it clockwise until it stops.
 - 8.2.4. Connect one end of the flexible gas tubing to the gas regulator.
 - 8.2.5. Connect the other end of the flexible gas tubing to the porous metal sparger.
 - 8.2.6. Insert the gas tubing nearest the sparger through one of the stopper holes.
 - 8.2.7. Place the sparger in the HPLC storage solution bottle and cap with the stopper.
- 8.3. Open the helium tank with the flow of helium turned off:
 - 8.3.1. Verify that the gas pressure is turned off by turning the regulator valve counter-clockwise for one half turn (in the direction marked “DECREASE”). The valve knob should turn freely.
 - 8.3.2. Close the delivery valve by turning it clockwise until it stops.
 - 8.3.3. Slowly open the tank valve by turning it counter-clockwise.
- 8.4. Dispense the solution to a correctly sized laboratory bottle:
 - 8.4.1. Select an empty laboratory bottle that has 20%-50% more volume than the quantity of solution to be sparged. (Note: additional volume is needed as head space to accommodate the sparger and bubbles without spilling.)
 - 8.4.2. Measure the desired amount of solution and transfer it to the selected bottle. Verify that the bottle is at least 50% full and no more than 80% full.
- 8.5. Place the sparger in the solution bottle:
 - 8.5.1. Remove the sparger from where it was stored in the HPLC storage solution bottle.
 - 8.5.2. Rinse the sparger with water from a wash bottle over a waste beaker.
 - 8.5.3. Place the sparger into the solution bottle and cap with the stopper.
 - 8.5.4. Verify that the sparger is submerged in the solution and that the unused stopper hole is unobstructed.

SOP: Degassing a Solution by Helium Sparge

- 8.6. Turn on the flow of helium and set the deliver pressure to 3 PSI:
 - 8.6.1. Verify that the gas pressure is turned off by turning the regulator valve counter-clockwise for one half turn (in the direction marked “DECREASE”). The valve knob should turn freely.
 - 8.6.2. Slowly open the delivery valve by turning it counter-clockwise.
 - 8.6.3. Watching the delivery pressure gauge, slowly increase the gas pressure by turning the regulator valve clockwise (in the direction marked “INCREASE”) until the delivery gauge reads 3 PSI.
 - 8.6.4. Observe bubbles rising from the sparger. Verify that there is sufficient head space in that bottle so that solution is not expelled from the bottle.
- 8.7. Sparge the solution for 15 minutes.
- 8.8. Turn off the flow of helium:
 - 8.8.1. Turn the gas pressure off by turning the regulator valve counter-clockwise for two full turns (in the direction marked “DECREASE”).
 - 8.8.2. Close the delivery valve by turning it clockwise until it stops.
- 8.9. Store the sparger:
 - 8.9.1. Remove the sparger from the solution bottle and cap the solution bottle.
 - 8.9.2. Rinse the sparger with water from a wash bottle over a waste beaker.
 - 8.9.3. Place the sparger in the HPLC storage solution bottle and cap with the stopper.
- 8.10. Close the tank valve by turning it clockwise until it stops.

SOP: Degassing a Solution by Helium Sparge

9. Attachments

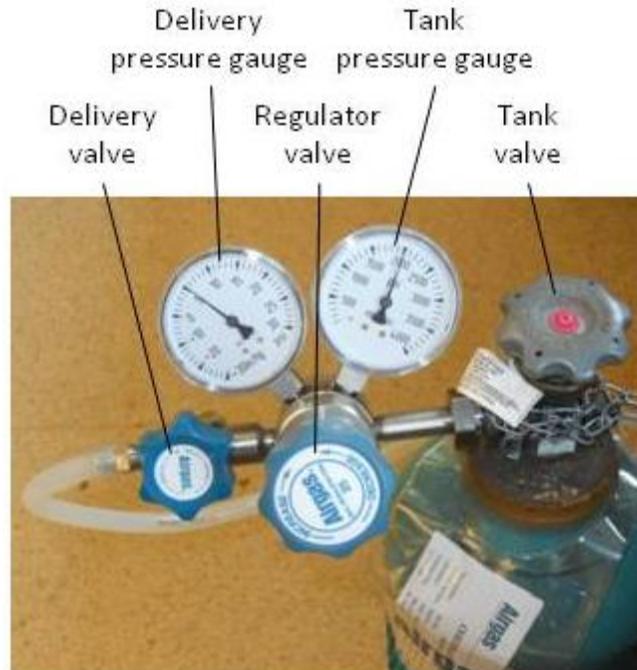


Figure 1. Helium Tank and Gas Regulator



Figure 2. Porous Metal Sparger

10. History

<i>Revision Number</i>	<i>Effective Date</i>	<i>Preparer</i>	<i>Description of Change</i>
0	09/25/2013	John Buford	Initial release

SOP: Buck Scientific BLC-20P HPLC Operation

Approvals

Preparer: John Buford

Date: 16OCT13

Reviewer: Tim Kull

Date: 30OCT13

Reviewer: Dr. Margaret Bryans

Date: 31OCT13

1. Purpose

- 1.1. Basic operation of the Buck Scientific BLC-20P isocratic HPLC system in order to assay a sample using reverse phase high performance liquid chromatography (RP-HPLC).

2. Scope and Applicability

- 2.1. High performance liquid chromatography (HPLC) is an analytical chemistry technique for separating the components of a liquid sample and for identifying and quantifying the components of the sample. This SOP provides the basic operations required to perform an assay using the Buck Scientific BLC-20P isocratic HPLC system, a reverse phase HPLC column, and a compatible mobile phase solution. Other process-specific SOPs are intended to provide the details of HPLC column selection, mobile phase solution preparation, sample preparation, flow rates, and run times.

3. Summary of Method

- 3.1. Prepare the mobile phase and storage solutions
- 3.2. Power up the HPLC system components and start the PeakSimple data collection software
- 3.3. Equilibrate the system with mobile phase solution
- 3.4. For each sample, run an assay:
 - 3.4.1. Use PeakSimple to start a new run
 - 3.4.2. Load and inject the sample
 - 3.4.3. Collecting data and store data
 - 3.4.4. Re-equilibrate the system if directed by the process-specific SOP
- 3.5. Wash the system with storage solution
- 3.6. Power down the system

4. References

- 4.1. SOP: Degassing a Solution by Helium Sparge
- 4.2. SUPPLEMENT to Operator's Manuals, BLC-20S PLUS Stack HPLC Systems, PN: DS090-0056, Rev A A333.

SOP: Buck Scientific BLC-20P HPLC Operation

5. Definitions

<i>CV</i>	Column Volume; the volume (mL) of the column containing the stationary phase; CV=2.91 mL for a standard size (4.6 X 250 mm) column
<i>Equilibration</i>	Running the mobile phase solution through the column prior to injecting the sample in order to bring the system into equilibrium
<i>Flow rate</i>	The rate (mL/min) that solution is pumped through the column. The operating flow rate is determined by the assay protocol, generally 1.0 mL/min for a standard size (4.6 X 250 mm) column
<i>Helium sparge</i>	Using a stream of helium bubbles to sweep dissolved air out of liquids (helium is virtually insoluble in most HPLC solvent solutions, so very little helium replaces the air)
<i>HPLC</i>	High Performance Liquid Chromatography
<i>Isocratic</i>	The composition of the mobile phase solution is constant; the system has only one pump.
<i>Mobile phase</i>	The solvent solution used to carry the sample through the column
<i>PeakSimple</i>	Software used to collect and display data
<i>PSI</i>	Pounds per Square Inch
<i>Reverse phase chromatography</i>	Separation based on hydrophobicity under conditions where the stationary phase is more hydrophobic than the mobile phase.
<i>Stationary phase</i>	The chromatography matrix through which the sample travels.

6. Precautions

- 6.1. Most solvents used for HPLC are toxic and flammable. For each solvent, read the Material Safety Data Sheet (MSDS) for hazards, handling and storage information. Wear personal protection equipment (PPE) and use a fume hood as required. Store solvents as indicated by the MSDSs.
- 6.2. HPLC systems operate at high pressures. Personnel injury and equipment damage can result if maximum pressure is exceeded or the pump runs dry. Monitor pressure readings and solution level whenever the pump is running. If pressure exceeds 2500 psi or if the solution runs out, stop the pump immediately by pressing the RUN/STOP button. Do not set the flow rate higher than 1.5 ml/min with a 250 mm column.
- 6.3. Different mobile phase solutions interact with the stationary phase differently, resulting in different back pressures for a given flow rate (see Table 3 for example pressure readings for various mobile phase solutions). When changing mobile phase solutions, monitor pressure readings carefully while running the first 5 CV of the new solution as it replaces the old solution in the lines and column.
- 6.4. Flow rate consistency is affected by the quality of the solutions. Use HPLC-grade solvents and filter solutions using a sub-micron filter (preferably 0.22 μ m). Degas solutions prior to use.
- 6.5. To avoid microbial growth, do not leave the system in a high aqueous solution for a prolonged period. The system should be washed with a storage solution of 50% Methanol/H₂O or 50% Acetonitrile/H₂O if it is to be idle more than a few hours.

SOP: Buck Scientific BLC-20P HPLC Operation

7. Responsibilities

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

8. Equipment and Materials

- 8.1. Buck Scientific BLC-20P HPLC system pre-configured with:
 - 8.1.1. UV-Vis detector
 - 8.1.2. Fluorometer (optional)
 - 8.1.3. PeakSimple Chromatography Data System
 - 8.1.4. Computer system with PeakSimple software installed
 - 8.1.5. Reverse phase HPLC column
- 8.2. HPLC-grade solvent for mobile phase solution
- 8.3. HPLC-grade methanol for storage solution
- 8.4. HPLC-grade water
- 8.5. Chemically compatible sub-micron filters (preferably 0.22 μm)
- 8.6. 2 laboratory bottles for mobile phase solution and waste
- 8.7. 2 laboratory bottles for storage solution and waste
- 8.8. Small bottle for mobile phase (to be used for cleaning the sample syringe)
- 8.9. Sample overflow waste beaker
- 8.10. 5 mL Luer-Lok syringe
- 8.11. 100 μL HPLC sample syringe
- 8.12. Parafilm
- 8.13. Timer

9. Procedure

Note: this BLC-20P isocratic HPLC is configured with one pump, a UV-Vis detector, an add-on fluorescence detector, and a four channel serial port connected to a computer running PeakSimple software. The pump and UV-Vis detector are controlled by the HPLC front panel. Data is collected and displayed by the PeakSimple software on the computer.

9.1. Prepare mobile phase and storage solutions:

- 9.1.1. A process-specific SOP should provide the composition and volume of mobile phase required. For an example, see Table 2. Example Solution Volume Calculations.
- 9.1.2. Prepare the mobile phase solution into a labeled laboratory bottle that is sized appropriately for degassing per the Degassing a Solution by Helium Sparge SOP. Filter the solution using a sub-micron filter (preferably 0.22 μm) that is chemically compatible.
- 9.1.3. Prepare a minimum of 300 mL 50% Methanol/H₂O or 50% Acetonitrile/H₂O storage solution into a labeled 500 mL laboratory bottle. Filter the solution using a sub-micron filter (preferably 0.22 μm) that is chemically compatible.
- 9.1.4. Degas both the mobile phase and storage solutions per the Degassing a Solution by Helium Sparge SOP.

SOP: Buck Scientific BLC-20P HPLC Operation

- 9.1.5. Transfer approximately 10 mL of mobile phase solution to a small labeled bottle to be used for rinsing the sample syringe.
- 9.1.6. Label an empty laboratory bottle as storage solution waste. Label another empty bottle as mobile phase solution waste.
- 9.2. ***Power up the HPLC system components and start the PeakSimple data collection software:***
 - 9.2.1. Power up the computer system and monitor, then login.
 - 9.2.2. Power up the pump unit using the switch located on the lower-right side in the back.
 - 9.2.3. Power up the data system unit using the switch located on the upper-left side in the back.
 - 9.2.4. Optionally, power up the fluorometer unit using the switch on the front.
 - 9.2.5. Launch the PeakSimple data collection software:
 - 9.2.5.1. Double click on the PeakSimple icon on the desktop. (Alternatively, navigate to C:\Peak426-32bit and run Peak426-32bit.exe.) The PeakSimple window should appear as the software automatically connects to the HPLC hardware.
 - 9.2.6. Allow the UV-Vis detector to warm up for 60 minutes prior to collecting data.
- 9.3. ***Switch the system to mobile phase solution:***
 - 9.3.1. Verify that the pump is off. The Run LED should be off.
 - 9.3.2. Place the intake line into the mobile phase solution bottle and cover with Parafilm. Verify that the frit is submerged in the solution.
 - 9.3.3. Place the outlet line into the mobile phase waste bottle.
 - 9.3.4. Place the sample overflow line into a small waste beaker.
- 9.4. ***Purge the intake line and prime the pump:***
 - 9.4.1. Attach an empty 5 mL Luer-Lok syringe to the purge valve.
 - 9.4.2. Open the prime/purge valve by turning it two full turns counter-clockwise.
 - 9.4.3. Watching the intake line for bubbles, slowly draw the syringe plunger until it is fully drawn. Mobile phase solution and bubbles should fill the syringe.
 - 9.4.4. Close the prime/purge valve by rotating it clockwise until it stops.
 - 9.4.5. Remove the syringe from the purge valve and expel the contents into the waste bottle.
 - 9.4.6. Repeat attaching the syringe to the purge valve, drawing bubbles and solution into the syringe, and expelling into the waste bottle until free of bubbles (generally 10- to-15 mL of mobile phase are needed).
- 9.5. ***Start the pump and gradually increase the flow rate to the operating rate:***
 - 9.5.1. A process-specific SOP should provide the flow rate for the mobile phase.
 - 9.5.2. Set the initial flow rate to 0.1 mL/min:
 - 9.5.2.1. Press the MODE button on the front panel repeatedly until the Flow LED turns on. The current flow rate (in ml/min) appears on the digital display.
 - 9.5.2.2. Press the Flow up arrow button to increase the flow rate setting and press the down arrow button to decrease the flow rate setting.
 - 9.5.2.3. Repeat pressing the Flow arrow buttons until 0.10 is displayed.
 - 9.5.3. Start the pump by pressing the RUN/STOP button. The Run LED should turn on.
 - 9.5.4. Monitor the pressure readings and solution level:

SOP: Buck Scientific BLC-20P HPLC Operation

- 9.5.4.1. Display the pressure reading by pressing the MODE button repeatedly until the Pressure LED turns on. The current pressure (in psi) appears on the digital display.
- 9.5.4.2. Verify that that solution is dripping into the waste bottle.
- 9.5.4.3. If pressure exceeds 2500 psi or if the solution runs out, stop the pump immediately by pressing the RUN/STOP button.
- 9.5.5. Gradually increase the flow rate in 0.1 mL/min increments over a period of 5 minutes to the specified flow rate:
 - 9.5.5.1. Press the MODE button on the front panel repeatedly until the Flow LED turns on. The current flow rate (in ml/min) appears on the digital display.
 - 9.5.5.2. Increase the flow rate setting by 0.1 mL/min by pressing the Flow up arrow button.
 - 9.5.5.3. Monitor the pressure readings and solution level.
 - 9.5.5.4. Repeat increasing the flow rate setting by 0.1 mL/min increments over a period of 5 minutes until the specified flow rate is achieved and pressure readings stabilize.
- 9.6. ***Set the UV-Vis detector wavelength and autozero the detector:***
 - 9.6.1. A process-specific SOP should provide the assay run time and the UV-VIS detector wavelength.
 - 9.6.2. Set the UV-Vis detector wavelength by pressing the λ up and down buttons on the front panel until the specified wavelength is displayed.
 - 9.6.3. Autozero the UV-Vis detector by pressing the AUTOZERO button on the front panel.
- 9.7. ***Equilibrate the system by running mobile phase:***
 - 9.7.1. A process-specific SOP should provide the equilibration run time. The run time may be expressed in terms of column volumes (CV); see Equation 1 for an example of converting a CV to a run time.
 - 9.7.2. Operate the pump to run for the specified run time. Monitor the pressure readings and solution level. Monitor detector values and notify the instructor if the values appear to be unstable.
 - 9.7.3. Alternatively, run an assay of a blank (see below) using mobile phase solution as the sample.
- 9.8. ***For each sample, run an assay:***
 - 9.8.1. Use PeakSimple to start a new run and edit the run time:
 - 9.8.1.1. Select File > New from the menu bar.
 - 9.8.1.2. Select Edit > Channels... from the menu bar. The Channels dialog box should appear.
 - 9.8.1.3. Select Channel 1: Details from the Channels dialog box. The Channel details dialog box should appear.
 - 9.8.1.4. Enter the run time in the End time box.
 - 9.8.1.5. Verify that the Remote start check box is checked.
 - 9.8.1.6. Close the Channel details and Channels dialog boxes.
 - 9.8.2. Autozero the UV-Vis detector by pressing the AUTOZERO button on the front panel.

SOP: Buck Scientific BLC-20P HPLC Operation

- 9.8.3. Load and inject the sample:
 - 9.8.3.1. Verify that the injector port handle is set to the “Load” position.
 - 9.8.3.2. Fill the HPLC sample syringe with 100 μ L of sample, using care to avoid bubbles in the syringe.
 - 9.8.3.3. Insert the syringe needle into the sample injection port. See Figure 4. Sample Injection Port with HPLC Syringe Attached.
 - 9.8.3.4. Depress the syringe plunger, using care to avoid introducing bubbles. (Often there is a small bubble at the base of the plunger. Watch carefully and stop depressing the plunger before the bubble is loaded into the injector port. It is OK to leave a few μ L of sample in the syringe.)
 - 9.8.3.5. Turn the injector port handle clockwise from the “Load” to the “Inject” position. Note that the PeakSimple has started displaying the elapsed run time in upper right corner.
 - 9.8.3.6. After 10 seconds, turn the injector port handle counter-clockwise from the “Inject” back to the “Load” position.
 - 9.8.3.7. Remove the syringe from the sample injection port.
 - 9.8.3.8. Rinse the syringe by filling it from the small bottle of mobile phase solution and expelling it into the mobile phase waste bottle at least three times.
- 9.8.4. Operate the pump for the specified run time. Monitor pressure readings and solution level. At the end of the run time, note that PeakSimple elapsed run time switches to STANDBY in upper right corner.
- 9.8.5. Use PeakSimple to view results and save the data to a chromatogram file:
 - 9.8.5.1. Select View > Results... from the menu bar. The Results dialog box should appear.
 - 9.8.5.2. Click the Copy button to copy the data.
 - 9.8.5.3. Paste the data into an Excel spreadsheet.
 - 9.8.5.4. Close the Results dialog box.
 - 9.8.5.5. Select File > Save as... from the menu bar. The Save as dialog box should appear.
 - 9.8.5.6. Enter a directory and a meaningful file name (e.g. operator initials, experiment name, and run number). Click the Save button.
- 9.8.6. Re-equilibrate the system if directed by the process-specific SOP.
- 9.8.7. Repeat this section for each sample.
- 9.9. **Stop the pump:**
 - 9.9.1. Press the RUN/STOP button. The Run LED should turn off and pressure readings should decrease gradually.
 - 9.9.2. Monitor the pressure until it decreases to less than 100 psi.
- 9.10. **Wash the system by running 5 CV of storage solution:**
 - 9.10.1. Switch the system to storage solution per the instructions in section 9.2.6 above.
 - 9.10.2. Purge the intake line and prime the pump per the instructions in section 9.4 above.
 - 9.10.3. Set the initial flow rate to 0.1 mL/min.
 - 9.10.4. Start the pump and gradually increase the flow rate to 0.5 mL/min over a period of 5 minutes.
 - 9.10.5. Operate the pump for 30 minutes. Monitor pressure readings and solution level.

SOP: Buck Scientific BLC-20P HPLC Operation

- 9.10.6. Stop the pump and allow the pressure to decrease to less than 100 psi.
- 9.11. **Power down the system:**
- 9.11.1. Exit PeakSimple by selecting File > Exit from the menu bar. A prompt should appear asking Save all before exiting? Click the No button.
- 9.11.2. Power down the pump unit using the switch located on the lower-right side in the back.
- 9.11.3. Power down the data system using the switch located on the upper-left side in the back.

Attachments

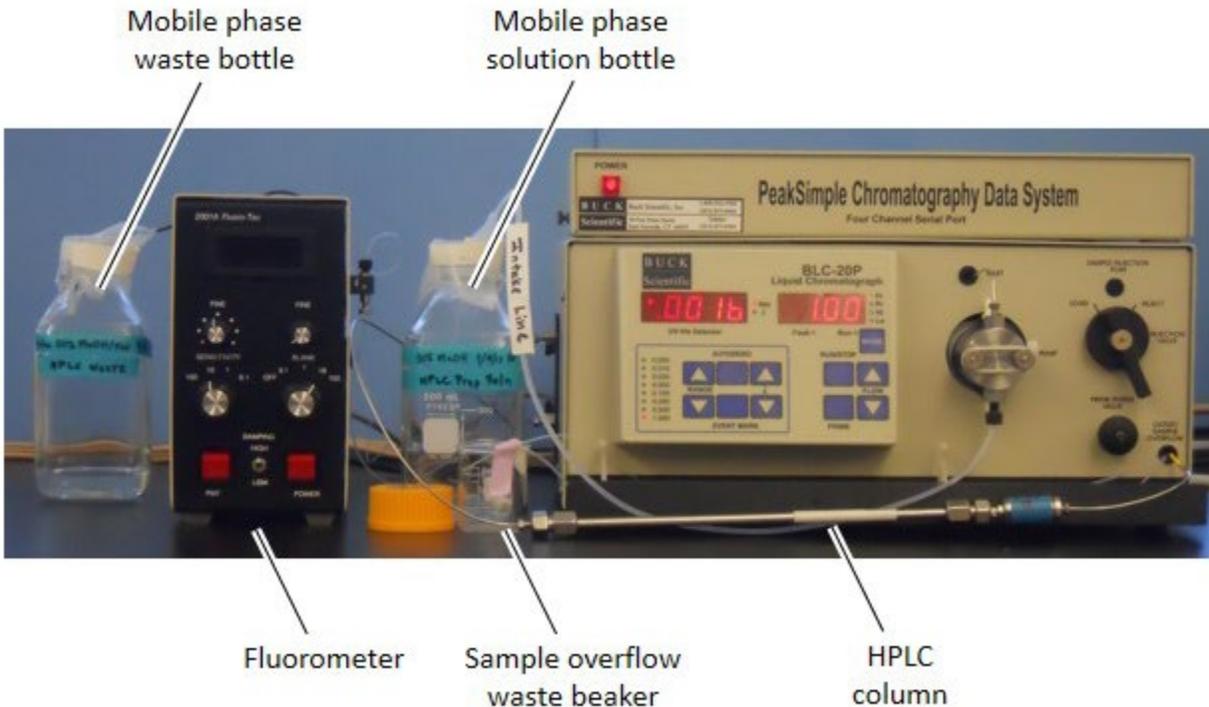


Figure 1. Buck Scientific BLC-20P HPLC System with Column and Fluorometer

SOP: Buck Scientific BLC-20P HPLC Operation

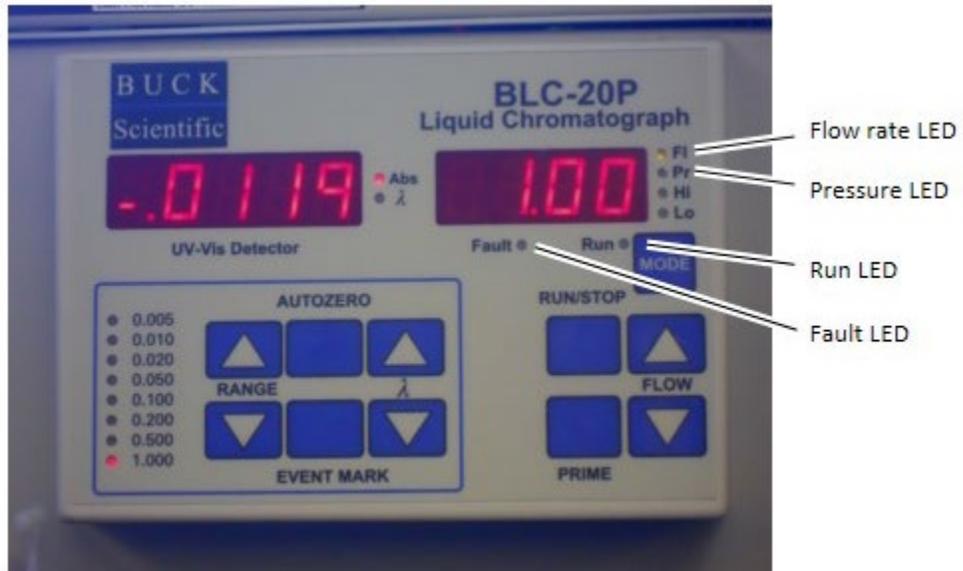


Figure 2. HPLC Front Panel

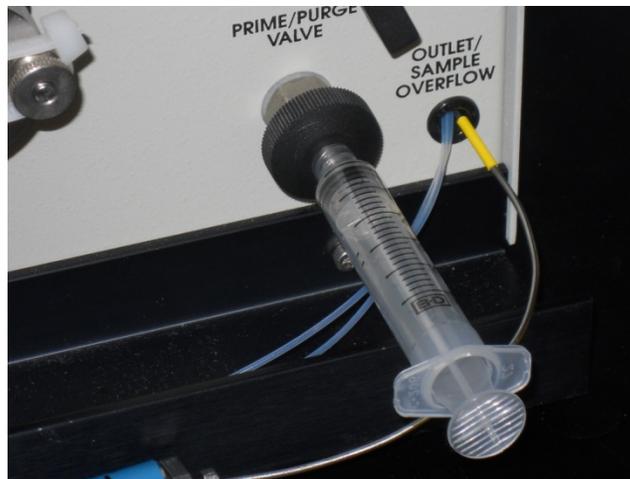


Figure 3. Purge Valve with Luer-Lok Syringe Attached

SOP: Buck Scientific BLC-20P HPLC Operation

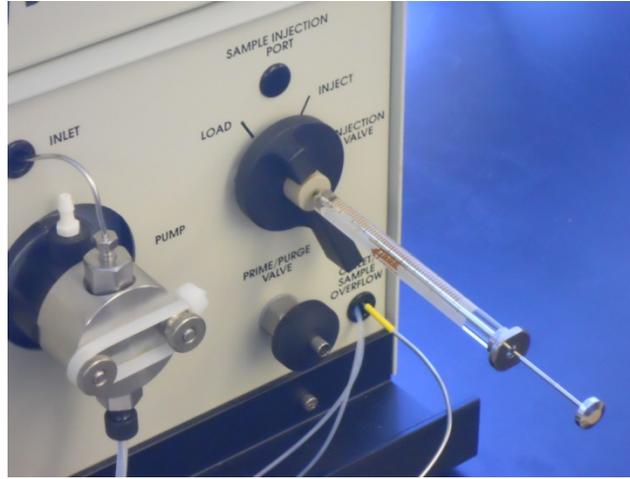


Figure 4. Sample Injection Port with HPLC Syringe Attached

Table 1. Common Column Void Volumes

Column dimension	Column void volume (mL)
250 x 4.6 mm	2.91
150 x 4.6 mm	1.74
100 x 4.6 mm	1.16
50 x 4.6 mm	0.58
250 x 4.0 mm	2.20
125 x 4.0 mm	1.10
250 x 2.0 mm	0.55
150 x 2.0 mm	0.33
50 x 2.0 mm	0.11

$$CV = \pi r^2 L$$

where:

CV = column volume in mL

r = column radius in cm

L = column length in cm

SOP: Buck Scientific BLC-20P HPLC Operation

Table 2. Example Solution Volume Calculations

Mobile Phase solution		
Operation	Calculation	Volume (ml)
Prime and purge	20 mL	20
Increasing flow rate	0.75 mL/min * 5 min	4
Initial equilibration	15 CV = 15 * 3 mL	45
Assays	N * flow rate * run time = 4 * 0.75 mL/min * 15 min	45
Syringe cleaning	10 mL	10
Re-equilibration	N * 5 CV = 4 * 3 mL	12
Reserve volume	100 mL	100
Subtotal	Sum of the above	236
Minimum volume	Subtotal * 120%	284

Storage solution		
Operation	Calculation	Volume (ml)
Prime and purge	20 mL	20
Increasing flow rate	0.5 mL/min * 5 min	3
Wash	15 CV = 15 * 3 mL	45
Reserve volume	100 mL	100
Subtotal	Sum of the above	168
Minimum volume	Subtotal * 120%	202

Equation 1. Example Run Time Calculations

Run volume = 15 CV
 Column volume (CV) = 3 mL
 Flow rate = 0.75 ml/min

$$\text{Run time} = \frac{\text{Run volume}}{\text{Flow rate}} = \frac{15 \text{ CV} * 3 \text{ mL/CV}}{0.75 \text{ mL/min}} = 60 \text{ min.}$$

Table 3. Example Flow Rates and Pressure Readings for a Haisil 100 C18 5µm 250 X 4.6mm Column

Mobile Phase Solution	Flow Rate (mL/min)	Pressure Readings (psi)
50% MeOH/H ₂ O	0.5	1400-1600
100% Isopropyl alcohol	0.5	1750-1950
100% H ₂ O	1.0	1600-1800
100% Acetonitrile	1.0	650-850
95% Hexane	1.0	500-700

SOP: Buck Scientific BLC-20P HPLC Operation

10. History

<i>Revision Number</i>	<i>Effective Date</i>	<i>Preparer</i>	<i>Description of Change</i>
0	31OCT13	John Buford	Initial release

Alternative Equipment

SOP: Isolation of Anti IL-8 mAb by Protein A Affinity Chromatography on the Bio-Rad Biologic LP Chromatography System

Approvals:

Preparer: Lara Dowland
Reviewer: Cianna Cooper

Date: 10JUN18
Date: 12JUN18

1. Purpose:

- 1.1. To isolate anti IL-8 mAb from conditioned medium (produced by CHO cells expressing recombinant anti-IL-8) using ultrafiltration and Protein A affinity chromatography with the Biologic LP Chromatography System.

2. Scope:

- 2.1. Applies to purification of mAb from prepared conditioned medium, which has been concentrated and its buffer exchanged by ultrafiltration.

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 (electronic)
- 4.2. BioRad Bio-Scale Mini Affi-Prep Protein A column -1 ml information booklet (BioRad)
- 4.3. Centricon 15 (30kDa cutoff) Concentrator Users Guide

5. Definitions: N/A

6. Precautions:

- 6.1. Routine care should be exercised in handling of buffers and samples of biological materials, which may have harmful biological activity in the case of accidental ingestion, needle stick, etc.
- 6.2. It is imperative that pumps never be allowed to run dry. Care must be taken to avoid air in the fluid path, which could damage the pumps or give spurious and uninterpretable readout from the UV and/or conductivity detectors.
- 6.3. Buffers must be degassed and filtered prior to use with the Biologic LP Chromatography System instrument.

7. Materials:

- 7.1. Biologic LP Chromatography system
- 7.2. BioRad Bio-Scale Mini Affi-Prep Protein A column -1 ml column; stored at 4°C- bring to room temperature prior to installation.
- 7.3. Additional Lab Equipment: balance, table top centrifuge w/ swinging bucket rotor
- 7.4. Lab Utensils: Beakers (250, 500ml, 1000 ml), 1 liter and 500 ml graduated cylinders
- 7.5. Reagents:
 - 7.5.1. 20 mM sodium phosphate buffer, pH 7.0
 - 7.5.2. 0.1 M citric acid, pH 3.0
 - 7.5.3. 1 M Tris base

SOP: Isolation of Anti IL-8 mAb by Protein A Affinity Chromatography on the Bio-Rad Biologic LP Chromatography System

- 7.5.4. Filtered deionized water (MilliQ or similar).
- 7.5.5. 20% Ethanol
- 7.5.6. 10N NaOH
- 7.5.7. Stock solutions of protease inhibitors: 10 mg/ml PMSF in isopropanol, 2 mg/ml leupeptin, 10 mg/ml aprotinin.

7.6. Lab Supplies:

- 7.6.1. Millipore Centricon 15 (30 kDa cutoff) centrifugal concentrators (2).
- 7.6.2. Filters (0.2 μ m); (3 bottle top; 2 syringe mounted)
- 7.6.3. Corning bottles for vacuum filtration, degassing of all chromatography buffers.
- 7.6.4. Syringe (20 ml) – (2).
- 7.6.5. Tubes for fraction collector – (27)
- 7.6.6. Graduated cylinders: 1L, 250 ml, 100 ml
- 7.6.7. Beakers: 1L, 400 ml, 200 ml

8. Procedure:

8.1. Prepare buffers and solutions

8.1.1. Buffer A: Binding buffer: 20 mM sodium phosphate, pH 7.0

- 8.1.1.1. Weigh 0.80 ± 0.02 gm NaH_2PO_4 and transfer to a 1 L flask with magnetic stir bar.
- 8.1.1.2. Weigh 3.60 ± 0.02 gm $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ and transfer to the same flask.
- 8.1.1.3. Measure 980 ml deionized water in a graduated cylinder and add the water to the solids in the flask.
- 8.1.1.4. Stir until the solids have dissolved, then adjust the pH to 7.0.
- 8.1.1.5. Transfer to a 1L graduated cylinder and adjust the final volume to 1L.
- 8.1.1.6. Sterile filter the solution, allowing it to degas for 15-20 minutes. Label appropriately.

8.1.2. Buffer B: Elution buffer: 0.1M citric acid, pH 3.0

- 8.1.2.1. Weigh 3.84 gm citric acid in a 400 ml beaker with magnetic stir bar.
- 8.1.2.2. Dissolve in 180 ml deionized water.
- 8.1.2.3. Adjust the pH dropwise with 10N NaOH, to a final pH of 3.0
- 8.1.2.4. Transfer the solution to a 250 ml graduated cylinder. Adjust the final volume to 200 ml.
- 8.1.2.5. Filter the solution, allowing it to degas for 15 – 20 minutes.
- 8.1.2.6. Label appropriately.

8.1.3. 1M Tris base: added to fraction collector tubes to rapidly neutralize acid-eluted fractions from the protein A column.

- 8.1.3.1. Weigh 12.11 gm Tris base [tris(hydroxymethyl)aminomethane] into a plastic weigh boat and transfer to a 200 ml beaker with a stir bar.
- 8.1.3.2. Measure 85 ml deionized water in a graduated cylinder and transfer the water to the beaker containing Tris powder. Stir until dissolved.
- 8.1.3.3. Transfer the Tris solution quantitatively to a 100 ml graduated cylinder, rinsing the beaker with small aliquots of water, which are then added to the cylinder until a final volume of 100 ml is obtained.

SOP: Isolation of Anti IL-8 mAb by Protein A Affinity Chromatography on the Bio-Rad Biologic LP Chromatography System

8.1.3.4. Filter the solution. Degassing is not necessary.

8.2. Sample Collection and Preparation

The operator will require up to 10 ml of sample per sample injection (see below). Conditioned medium from a late log phase/early stationary phase culture of cells producing the desired mAb in a low IgG medium is an excellent source.

8.2.1. Transfer conditioned medium to centrifuge bottles; centrifuge at 1000 x g for 10 minutes at 4°C.

8.2.2. Decant the supernatant into a beaker (for smaller volumes that are to be filtered with a syringe-mounted filter) or directly into a bottle top 0.2µm filter. Filter the supernatant into a 50 ml conical tube or bottle.

8.2.3. Transfer 15 ml of the filtered supernatant into each of two Centricon 15 (30kDa) ultrafiltration devices. Centrifuge in the table top centrifuge with swinging bucket rotor at 3500 x g, 4°C for 25 minutes.

8.2.4. Remove the filter insert, pour off the filtrate in the bottom tube and return the insert to the tube. Add additional CM to fill the upper chamber and repeat centrifugation.

8.2.5. Repeat step 9.2.4 until sufficient quantity (50-75 ml of starting material) of the supernatant is concentrated.

8.2.6. Resuspend the concentrate in buffer A and repeat centrifugation.

8.2.7. Remove the concentrate with a 200µl or 1000µl pipette and place in a small beaker. Rinse the ultrafiltration membrane multiple times with 1 ml aliquots of buffer A (at room temperature) and combine the rinses with the concentrate in the beaker, until a volume of approximately 10 ml has been reached.

8.2.8. Mix the concentrate and rinses, then draw into a 10 ml syringe. Mount a 0.22µm filter on the syringe and pass the mAb-containing solution through it into a clean beaker.

8.2.9. The sample must be at room temperature prior to application to the column.

8.3. Start-up and preparation of Biologic LP Instrument and computer:

Degassed buffers should be in place prior to turning on the Biologic LP instrument. Equipment start-up requires turning on the instrument and, separately, the computer connected to it.

8.3.1. Place the degassed buffers A and B on top of the Biologic LP instrument.

8.3.2. Locate Inlet tubing A and B and C (atop the instrument and resting in water or 20% ethanol).

8.3.3. Transfer tubing Inlet A to the buffer A bottle.

8.3.4. Transfer tubing Inlet B to the buffer B bottle.

8.3.5. Transfer tubing Inlet C to the 15ml Falcon tube in a rack containing the sample to be purified.

8.3.6. The On/Off switch for the instrument is located on the lower left front. Switch to the 'On' position.

8.3.7. Turn on the computer.

SOP: Isolation of Anti IL-8 mAb by Protein A Affinity Chromatography on the Bio-Rad Biologic LP Chromatography System

- 8.3.8. Login to the computer using credentials provided by the College. Open the LP Data View Software.
- 8.3.9. Confirm that the correct column (BioRad Protein A-HP 1 ml) is attached to the system. If not, refer to SOP2.00 BioLogic LP Chromatography System Operating SOP for directions on connecting the column.
- 8.3.10. Prepare the fraction collector for later steps by filling the carousel with clean tubes (28). Add 200 μ l 1M Tris to the bottom of each tube – this serves to rapidly neutralize the acidic eluent (which destabilizes some antibodies).
- 8.3.11. Place all ‘Waste’ tubing into a 500ml Erlenmeyer flask.
- 8.3.12. Place the 15ml Falcon tube containing the sample to be purified in a rack on top of the platform.
 - 8.3.12.1. Place Buffer line C into the falcon tube. This will serve to inject the sample rather than using a loop.
- 8.4. Performing a chromatography run:**
 - 8.4.1. Press the List of Methods softkey, select the method with file name “Anti IL8”.
 - 8.4.2. Press the Run mode softkey.
 - 8.4.3. Press Record on the LP Data View software.
 - 8.4.4. Observe that the fraction collector is receiving drops.
 - 8.4.5. Monitor the computer screen for error messages or warnings.
 - 8.4.6. Allow the method to run to completion, at which time the system will be re-equilibrated and ready for subsequent runs.
 - 8.4.7. Remove tubes from the fraction collector and place in a rack for storage at 4°C, awaiting further analysis. Cover the top of the tubes with lab film. The peak of absorbance at 280 nm which eluted with the low pH buffer B contains purified mAb, which will be examined by the QC Biochemistry Dept.
- 8.5. Equipment shut-down and short term (less than 3 days) storage**
 - 8.5.1. After completion of the final separation of the day, transfer Inlet tubing A, B and C to a flask of degassed deionized water (250ml or greater).
 - 8.5.2. Refer to SOP2.00 Biologic LP Chromatography System Operating SOP to clean the lines.
 - 8.5.3. Turn off the instrument.
- 8.6. Equipment shut-down and long term (3 days or more) storage**
 - 8.6.1. After completion of the System Short Term Storage method, transfer Inlet tubing A, B and C to a flask of degassed 20% ethanol (250ml or greater).
 - 8.6.2. Refer to SOP2.00 Biologic LP Chromatography System Operating SOP to clean the lines.
 - 8.6.3. Remove the BioRad Protein A-HP column from the instrument and cap both ends, taking care to avoid introduction of air into the column. Store the column in a refrigerator.
 - 8.6.4. Turn off the Biologic LP instrument.
- 8.7. Printing Your Chromatogram**
 - 8.7.1. Under File, choose to Print (or Save as PDF to use a different printer).

SOP: Isolation of Anti IL-8 mAb by Protein A Affinity Chromatography on the Bio-Rad Biologic LP Chromatography System

9. **Attachments:** N/A

10. History:

Name	Date	Amendment
Lara Dowland	12Jun18	Initial Release

SOP: BioLogic LP Chromatography System Operation

Approvals:

Preparer: Lara Dowland
Reviewer: Cianna Cooper

Date: 10JUN18

Date: 12JUN18

1. Purpose:

1.1. Operation of the BioLogic LP Chromatography System.

2. Scope:

2.1. 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:

6. Precautions: N/A

7. Materials:

7.1. >250ml Deionized Water

7.2. ~500ml Buffer A (Refer to the appropriate chromatography SOP)

7.3. ~500ml Buffer B (Refer to the appropriate chromatography SOP)

7.4. ~500ml Cleaning Solution (Refer to the appropriate chromatography SOP)

7.5. Container for Waste Fluid

7.6. Collection Tubes for Fraction Collector or collection containers

7.7. Column

7.8. Resin (Refer to the appropriate chromatography SOP)

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. Programming the Biologic LP Chromatography instrument

8.5.1. To enter program mode, press the **Prog** key. It is usually easier to modify an existing program than to program a new one. To modify an existing program, go to Step 8.5.9.

8.5.2. Select the **New Method** soft key.

8.5.3. Select **Time** or **Volume mode**. These are the units used to define actions within a method; once the selection is made it cannot be changed.

8.5.4. Press the **Add** soft key. Enter the desired parameters for duration, buffer and flow rate. Note – to program the Fraction Collector or Alarm without returning to Method list, press the **Collector** and **Alarm** soft keys respectively.

SOP: BioLogic LP Chromatography System Operation

8.5.4.1. Select Buffer – buffers are indicated by the letters available. The brackets around one the buffers [], indicates which buffer is currently selected. Use the **Prev** and **Next** keys to change the selection. **Grad** key is used to program a linear gradient in this step. **Mix** is used to program a mixture in this step. **Cancel** cancels the addition or modification of this step and returns to the Pump Table as it was originally. **OK** confirms the selected buffer as indicated by the brackets surrounding the letter and continues to the next step. The letters G and M stand for gradient and mixture. Gradient represents a linear gradient made from Buffers A and B and Mixture refers to a single solution made by mixing Buffers A and B.

8.5.4.2. Length of Step- the duration of the step is entered using the number keys. The duration is in units of time (minutes) or volume (ml).

8.5.4.3. Flow Rate – flow rate of this step is entered using the number soft keys. This is the final entry for a pump step.

8.5.4.4. Continue entering buffer steps.

8.5.5. Press the **Collector** instrument key while in Program mode. Select Collection mode from the following choices:

All – collect all mode collect fractions throughout the method.

Threshold – collect peaks by defining a threshold value in absorbance units (AU).

Windows – allows you to specify periods called “windows” within the method when fractions will be collected.

Thresh+Win – selects threshold plus collection windows.

8.5.5.1. Fraction Collection Summary – shows the current collection mode and an estimate of the number of fractions which will be collected. **Edit** key allows the fraction collection parameters to be viewed and changed. **Erase** key removes the existing fraction collection programming from the method. **New Mode** allows the collection mode to be changed by displaying the Select Collection Mode screen. **OK** returns to the Method List.

8.5.6. Press the **Alarm** key to set alarms during the program. Up to three alarms can be programmed in a method. Units will be either time or volume, depending on the choice made when the method was created.

8.5.6.1. The selected alarm is shown with an arrow (>) pointing to the alarm.

Use the **Prev** and **Next** keys to move the selection up and down the list.

Edit allows the parameters of the selected alarm to y changed. **Delete** removes the selected alarm from the table (* the alarm cannot be retrieved).

Add allows a new alarm to be added at the end of the table. The next time the Alarm Table is viewed, the alarms will be rearranged

in the order of occurrence. **OK** indicates the table is complete and returns to the Methods List.

SOP: BioLogic LP Chromatography System Operation

8.5.6.2. Alarm Settings – enter the time or volume for the alarm to occur using the number keys.

8.5.6.2.1. Hold On – programs the method to hold when the alarm occurs.

8.5.6.2.2. Hold Off – programs the method to run normally with the alarm occurs.

8.5.6.2.3. Cancel – returns to the alarm table without adding or modifying the alarm.

8.5.6.2.4. OK – returns to the Alarm Table with the new or modified alarm in the table.

8.5.7. Entering a Name for the New Method – method names can consist of up to 12 character and can include upper case letters, number and spaces.

8.5.7.1. Method Name Entry screen – allows method names to be entered. The underscore shown within the method name on the top line is the insertion point where the next character will be placed. Use the **Prev** and **Next** keys to move the insertion point. To enter a letter character, select the soft key which shows the range containing the desired letter followed by the number key indicated after selecting the range. To use a number, press the number key directly. Use the decimal (.) key to place a space in the name. Press the **Done** key to return to the List of Methods after the name has been entered.

8.5.8. Viewing and Editing a Method – press the **View Method** key; the Method List will be displayed. Each step of the method can be viewed and edited to make whatever changes are necessary. When the contents of the method are correct, press the **Done** key. If changes were made, there will be a prompt to save the method. Refer to step 8.5.8 to save the method with a new name.

8.5.8.1. The numbered steps indicate the pump step within the method. Below each step are any fraction collection events or alarm events which will occur during the step. Use the **Prev** and **Next** keys to scroll through the list of steps. Press the **Edit** softkey to make changes.

8.6. Pump Calibration

8.6.1. Based on the desired flow rate, select the appropriate tubing for the pump.

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.

8.6.2. Verify that the correct tubing is in the pump

8.6.2.1. Remove the platen by lifting the grey handle (figure 2).

8.6.2.2. If necessary, insert the correct tubing.

8.6.2.3. Replace platen and lock into place.

8.6.2.4. If tubing was replaced readjust the platen and recalibrate the pump

8.6.2.4.1. Loosen the platen adjust screw located on the top of the pump (fig. 2) by turning counterclockwise until there is slight resistance.

SOP: BioLogic LP Chromatography System Operation

- 8.6.2.4.2. 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
- 8.6.2.4.3. Recalibrate the pump
 - 8.6.2.4.3.1. Press the MANUAL mode key
 - 8.6.2.4.3.2. Press the PUMP instrument key
 - 8.6.2.4.3.3. Select FLOW, then select CALIBRATE
 - 8.6.2.4.3.4. Select the appropriate tubing size.
 - 8.6.2.4.3.5. Select NOMINAL.
- 8.7. **Purge the System** – this is done without the column attached.
 - 8.7.1. Place each buffer line into a container filled with deionized water or buffer.
 - 8.7.2. Attach the column inlet tube directly to the column outlet tube.
 - 8.7.3. Press the MANUAL mode key.
 - 8.7.4. Select BUFFER, starting with A.
 - 8.7.5. Select OK.
 - 8.7.6. Select PURGE.
 - 8.7.7. Allow system to purge until conductivity reading on the display panel of the controller stabilizes (less than 5 minutes).
 - 8.7.8. Select BUFFER.
 - 8.7.9. Using the arrow key, select B.
 - 8.7.10. Select OK.
 - 8.7.11. Allow system to purge until conductivity reading on the display panel of the controller stabilizes (less than 5 minutes).
 - 8.7.12. Select Buffer. Select C.
 - 8.7.13. Select OK.
 - 8.7.14. Allow system to purge until conductivity reading on the display panel of the controller stabilizes (less than 5 minutes).
 - 8.7.14.1. While the system is running, zero the UV Monitor.
 - 8.7.14.2. Press the UV instrument key.
 - 8.7.14.3. Select ZERO.
 - 8.7.14.4. Verify that the absorbance changes to zero on the display panel of the controller.
 - 8.7.14.5. Press the PUMP instrument key.
 - 8.7.14.6. Select STOP.
- 8.8. **Attach the Column/Equilibration of the Column**
 - 8.8.1. Disconnect the column inlet and outlet tubing from the tubing connector.
 - 8.8.2. Attach the column inlet tubing from the injector valve to the top of the column.
 - 8.8.3. Attach the column outlet tubing to the bottom of the column.
 - 8.8.4. Place tubing from the 3-way valve side port to the waste container.
 - 8.8.5. Press MANUAL mode key

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- 8.8.6. Select PURGE
- 8.8.7. Select START
- 8.8.8. Allow buffer to drip into the waste container from the side port until air bubbles are completely absent in the inlet tubing.
- 8.8.9. Select STOP.
- 8.9. Run the Column**
 - 8.9.1. Place all lines in the appropriate buffers/solutions as per the appropriate chromatography SOP.
 - 8.9.2. Press the PROGRAM mode key
 - 8.9.3. Select LIST METHODS
 - 8.9.4. Using the arrow keys, select the correct method as per the appropriate chromatography SOP.
 - 8.9.5. Select OPEN
 - 8.9.6. Using the arrow keys, verify that the method has not been changed.
 - 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.
- 8.10. Clean the Column**
 - 8.10.1. Place buffer lines into the appropriate cleaning solution (refer to the Isolation of anti IL-8 mAb by Protein A Affinity Chromatography SOP).
 - 8.10.2. Run the appropriate cleaning method (refer to Isolation of anti IL-8 mAb by Protein A Affinity Chromatography SOP).
- 8.11. Clean and Store the System**
 - 8.11.1. If the system will be used again with the same column within a few days, it may be stored as is after a run.
 - 8.11.1.1. Turn off the system and turn off the computer
 - 8.11.2. If the system will not be used within a few days it must be flushed with water then 20% ethanol.
 - 8.11.2.1. Disconnect the column
 - 8.11.2.2. Attach the column inlet tube directly to the column outlet tube.
 - 8.11.2.3. Place each buffer line into a container filled with deionized water.
 - 8.11.2.4. Press the MANUAL mode key
 - 8.11.2.5. Select BUFFER.
 - 8.11.2.6. Select MIX
 - 8.11.2.7. Type in 50% B
 - 8.11.2.8. Select OK
 - 8.11.2.9. Select PURGE.
 - 8.11.2.10. Allow system to purge until conductivity reading stabilizes (less than 5 minutes).

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- 8.11.2.11. Select BUFFER
- 8.11.2.12. Using the arrow key, select C
- 8.11.2.13. Select OK
- 8.11.2.14. Allow system to purge until conductivity reading stabilizes (less than 5 minutes).
- 8.11.2.15. Select STOP
- 8.11.2.16. Repeat steps 8.11.2.3 through 8.11.2.15 using 20%ethanol.

9. Attachments:

- 9.1. Figure 1: Controller Front Panel
- 9.2. Figure 2: Controller Pump
- 9.3. Figure 3: Photograph of the system with parts labeled

10. History:

Name	Date	Amendment
Lara Dowland	12Jun18	Initial release

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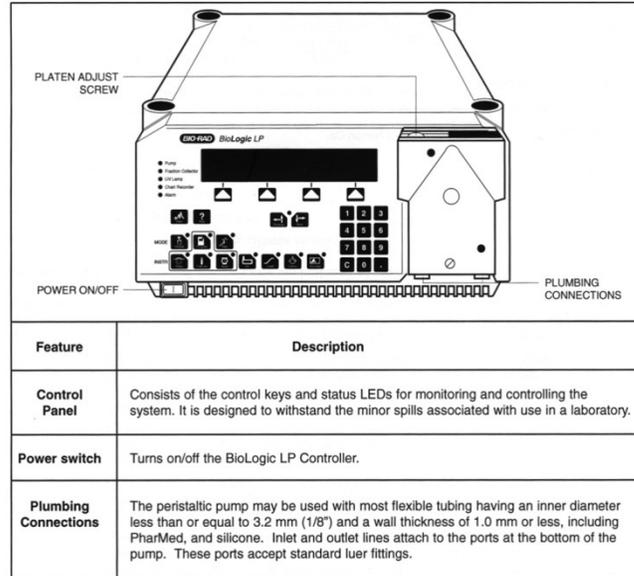


Figure 1: Controller Front Panel

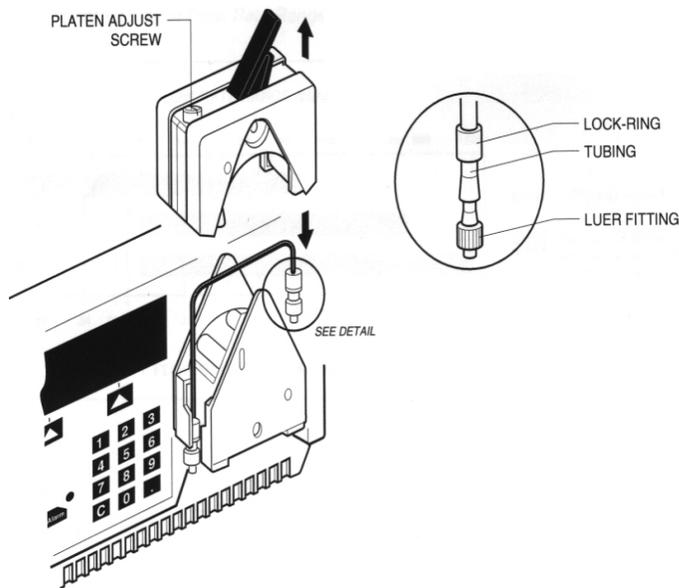


Figure 2: Controller Pump

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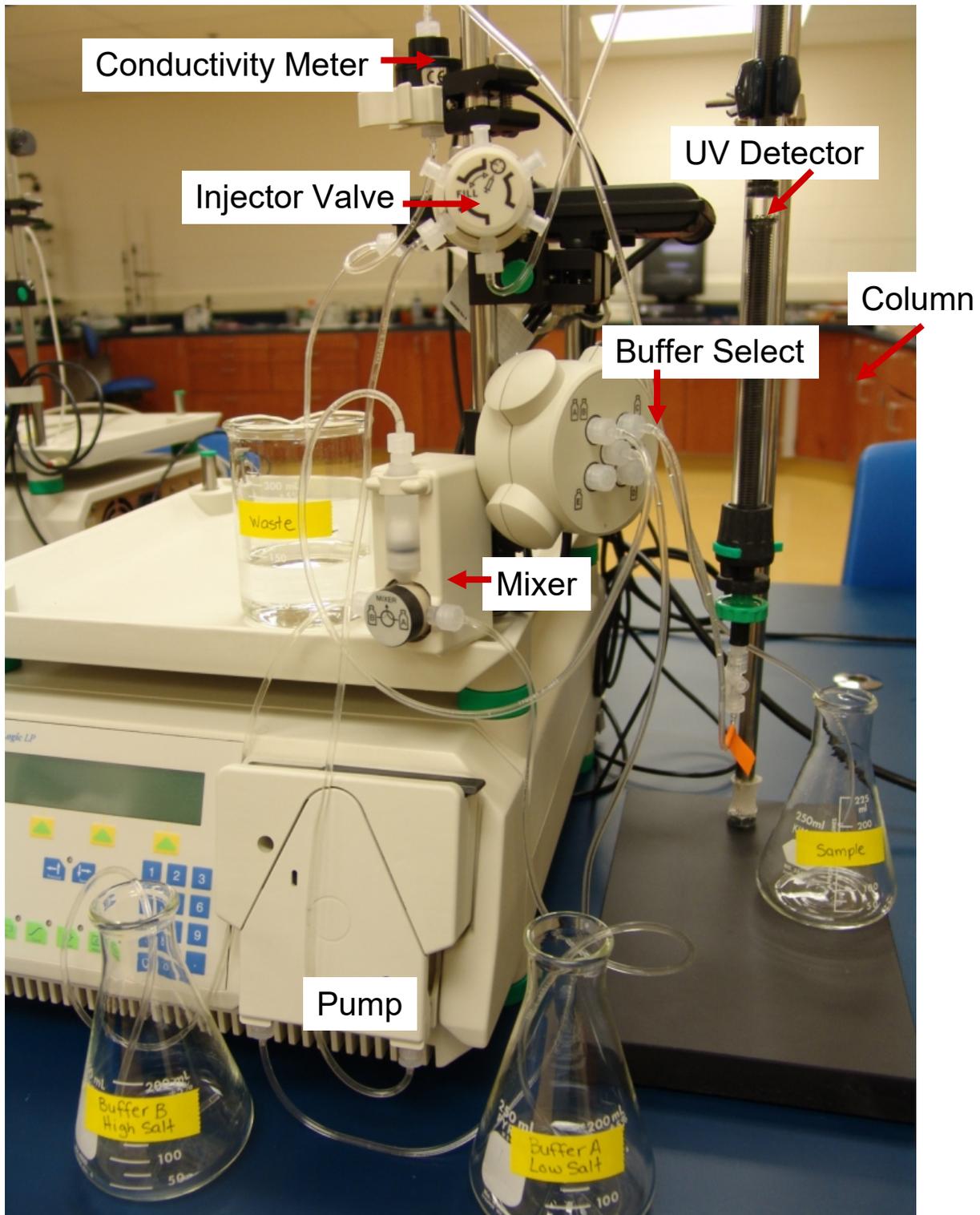


Figure 3: Photograph of the system with parts labeled

