Batch Record for Downstream Processing of Anti IL-8 mAb

Approvals:

Preparer: Jason McMillan & Dr. David Frank — Date: 15APR16
Reviewer: Hetal Doshi — Date: 08JAN20
Reviewer: Dr. Margaret Bryans — 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 peform 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 Filteration.

1.2 References

Title	*Doc #*
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

Equipment Type	*Manufacturer, Model*	*ID #*	*Initials/Date*	*Verifier/Date*
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

Component	*Quantity Required*	*Quantity Used*	*Initials/Date*	*Verifier/ Date*
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

Solution	*Volume*	*Date Prepared*	*Initials/ Date*	*Verifier/ Date*
0.1N NaOH (sodium hydroxide)				
0.05N NaOH (sodium hydroxide)				
TFF Buffer A: Binding buffer for Buffer exchange: 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 (phenylmethylsulfonylflouride), 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

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

Batch Record for Downstream Processing of Anti IL-8 mAb

3	Measure out the volume of Milli-Q water necessary to produce the desire concentration and transfer to the beaker, according to the formula: $Vol, ml = x \div 40 \div 0.1 \times 1000,$ where x = g NaOH measured Record the following: NaOH measured: _____ g Volume: _____ml		
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		

Batch Record for Downstream Processing of Anti IL-8 mAb

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		

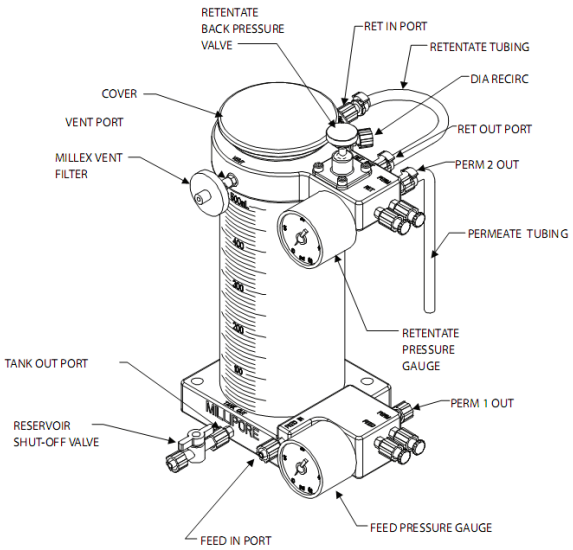
	Solution	*Initials/Date*	*Verifier/ Date*
	Leupeptin, 2 mg/ml		
1	Obtain a 5 mg vial of leupeptin. Open carefully with gloved hands in a fume hood.		
2	Pipet 2.5 ml MilliQ water into the vial and mix.		
3	Transfer in aliquots of 100 µl to several 1.5 ml tubes.		
4	Store the solution at 4ºC for one week or, preferably at -20ºC 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 4ºC for one week or, preferably at -20ºC. Aprotinin is stable at -20ºC for at least 6 months.		
	PMSF, 10 mg/ml in isopropanol		
	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.		
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 PMSF ÷ 10 mg/ml Record the following: PMSF: _____ mg Isopropanol: _____ml		
5	Mix to dissolve the powder		
6	Label the tube and store it at 4ºC. PMSF is stable in isopropanol (has a very short half life in aqueous solutions).		

1.6.2. Preparation of the Labscale TFF System

	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.	



#	*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.		

1.6.3. Flushing the Pellicon cassette.

#	*Task*	*Initials/ Date*	*Verifier/ Date*
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.		

Batch Record for Downstream Processing of Anti IL-8 mAb

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 an 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%).		

1.6.6.Concentration of Anti IL-8 mAb in conditioned medium

#	*Task*	*Initials/ Date*	*Verifier/ Date*
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

#	*Task*	*Initials/ Date*	*Verifier/ Date*
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 reads10 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 ed 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 -20ºC.		

1.6.8. Cleaning the Pellicon XL cassette ultrafiltration membrane.

Cleaning of the Pellicon cassette and its internal ultrafiltration membrane is achieved by:
1. flushing the system with MilliQ water (a repeat of procedure 1.6.3)
2. cleaning with 0.1N NaOH.
3. flushing once more with MilliQ water (procedure 1.6.3).
Cleaning may be initiated and left to continue while the subsequent operation (chromatography) is performed.

#	*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 reads10 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

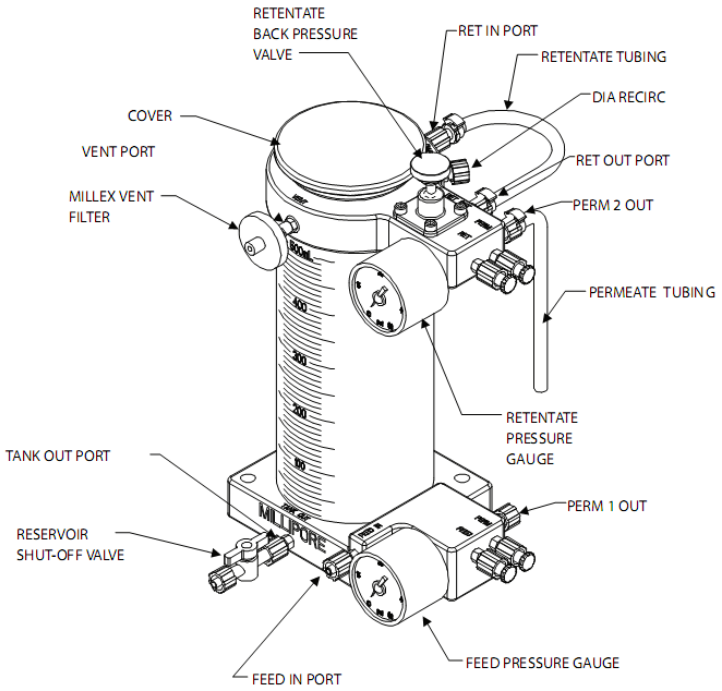


Figure 1: Reservoir Set Up
(http://www.millipore.com/userguides.nsf/docs/p60085)

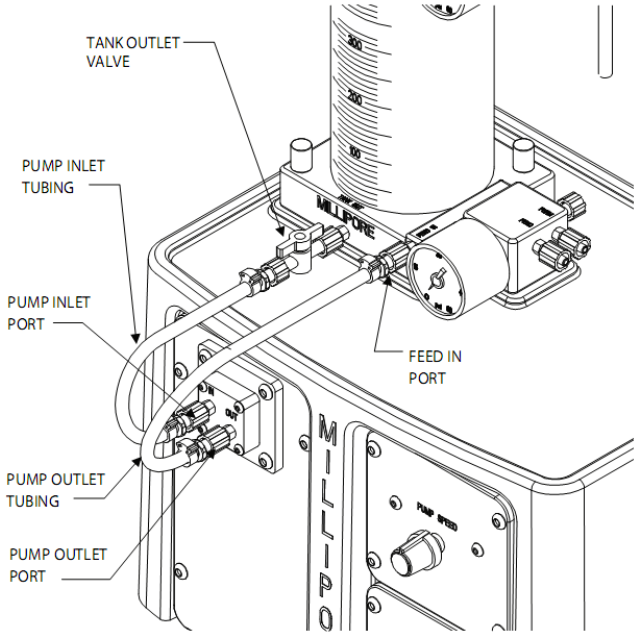


Figure 2: Pump Base Set Up

Batch Record for Downstream Processing of Anti IL-8 mAb

(http://www.millipore.com/userguides.nsf/docs/p60085)

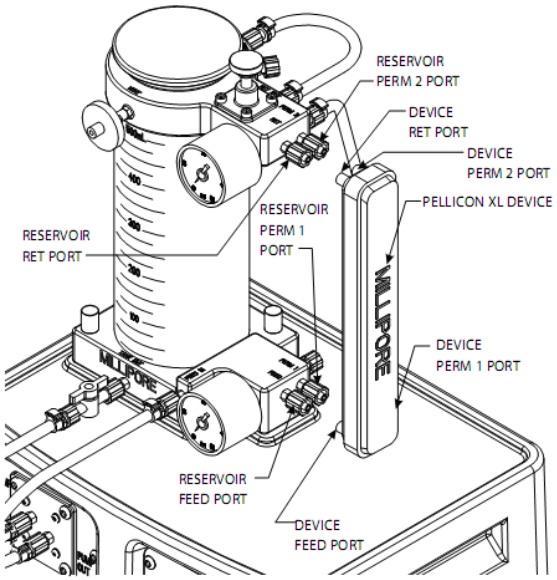


Figure 3: Installation of Pellicon XL Device
(http://www.millipore.com/userguides.nsf/docs/p60085)

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

Title	*Document Number*
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

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

2.4.Materials

Component	*Quantity Required*	*Quantity Used*	*Initials/Date*	*Verifier/Date*
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 fliter unit	4			

Batch Record for Downstream Processing of Anti IL-8 mAb

2.5. Solutions

Solution	*ID*	*Date Prepared*	*Volume Required*	*Volume Used*	*Initials/ Date*
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

#	*Task*	*Initials/Date*	*Verifier/Date*
	<u>Buffer A: Binding buffer</u>: 20 mM sodium phosphate, pH 7.0		
1	Weigh 1.084 ± 0.02g NaH_2PO_4 and transfer to a 1200ml beaker with magnetic stir bar.		
2	Weigh 3.273 ± 0.02g Na_2HPO_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.		
	<u>Buffer B: Elution buffer:</u> 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		

Batch Record for Downstream Processing of Anti IL-8 mAb

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 ***Current value*** is stable, click the ***Calibrate*** button.		
8	The calibration date and time are displayed in the dialog, along with values for ***Calibrated electrode slope*** (should be ≥ 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.		

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

#	*Task*	*Initials/Date*	*Verified Initial/Date*
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 4ºC for later analysis.		
5	Repeat the step 6.1 and 6.5 with remaining sample		

Batch Record for Downstream Processing of Anti IL-8 mAb

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	*Optional:* 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	*Optional.* Determine protein content per fraction by Bradford Protein Estimation. Refer to the SOP for that procedure.		
4	*Optional* Use Operations\Fraction Histogram to indicate average protein content per fraction.		
5	*Optional.* Use Operations\Activity Histogram to enter □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

Revision Number	*Effective Date*	*Preparer*	*Description of Change*
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