Batch Record for Downstream Processing of Anti-IL8 mAB: TFF Operation

Approvals:

Preparer: Jason McMillan & Dr. David Frank — Date: 15APR16
Reviewer: Hetal Doshi — Date: 18DEC18
Reviewer: Dr. Margaret Bryans — Date: 20DEC18

1.0 Description

This batch record directs and documents the isolation of Anti-IL8 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-IL8 mAb in conditioned growth medium by tangential flow filtration

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

2.0 References

Title	*Doc #*
Millipore Tangential Flow and Diafiltration Using Pellicon XL Device of tPA SOP	DP 1
SOP: End-of-Run Anti-IL8 mAb Process: Harvest, Centrifugation, Concentration,	DP
URL for Lab scale User guide and Documentation: http://www.emdmillipore.com/Web-US-Site/en_CA/-/USD/ViewParametricSearch-SimpleOfferSearch?SearchTerm=+labscale++pellicon&SelectedSearchResult=SFDocumentSearch&SearchContextPageletUUID=	N/A

4.0 Equipment

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

3.0 Components

Component	*Quantity Required*	*Quantity Used*	*Initials/Date*	*Verifier/ Date*
250ml Nalgene centrifuge bottles	3-4			

Batch Record for Downstream Processing of Anti-IL8 mAB: TFF Operation

Bottle-top vacuum 0.22 µm filtration device	1			
250ml Corning bottles	3-4			
10mlgraduated cylinder	1			
25ml beaker	1			
50ml beaker	1			
Nalgene Oak Ridge centrifuge tubes	2-4			

4.0 Solutions

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

5.0 Procedure:

5.1 Preparation of Solutions

	Solution	*Initials/Date*	*Verifier/ Date*
Step	**0.1N NaOH**		
1	Weigh approximately 2.5g NaOH		
2	Transfer the solid NaOH to a 600ml beaker with stir bar.		
3	Measure out the volume of Milli-Q water necessary to produce the desire concentration and transfer to the beaker, according to the formula:		

Batch Record for Downstream Processing of Anti-IL8 mAB: TFF Operation

	$Vol,ml = x \div 40 \div 0.1 \times 1000,$ where x = g NaOH measured Record the following: NaOH measured: _____g Volume: _____ml		

	0.05N NaOH			
1	Pipet 5ml MilliQ water into a 15ml plastic conical tube with screw cap.			
2	Pipet 5ml 0.1N NaOH into the same tube, cap, mix and label appropriately.			
	10% w/v Tween 80			
1	Measure approximately 80ml MilliQ water and a magnetic stir bar into a 200ml beaker.			
2	Place the beaker on a balance and tare the balance when stable.			
3	Pour 10g 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 100mlgraduated 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 100ml.			
7	Store the solution in an appropriately labeled bottle at room temperature.			

Batch Record for Downstream Processing of Anti-IL8 mAB: TFF Operation

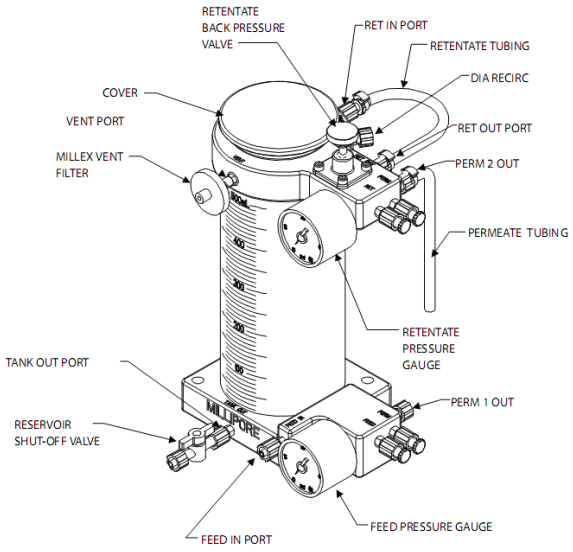
	Solution	*Initials/Date*	*Verifier/ Date*
	20mM Sodium Phosphate Buffer, pH 7.0 with 0.1% Tween 80		
1	Weigh 1.08 ± 0.02g NaH_2PO_4 and transfer to a 1200ml beaker with magnetic stir bar.		
2	Weigh 3.20 ± 0.02g $Na_2HPO_4 \bullet 7H_2O$ and transfer to the same flask.		
3	Measure 980ml MilliQ water in a graduated cylinder and add the water to the solids in the flask.		
4	Pipet 10ml of 10% w/v Tween 80 into the flask.		
5	Stir until the solids have dissolved, check the pH and if needed adjust the pH to 7.0 with 1M phosphoric acid. Bring to the volume to 1L		
6	Sterile filter the solution and label appropriately.		
	Leupeptin, 2mg/ml		
1	Obtain a 5mg vial of leupeptin. Open carefully with gloved hands in a fume hood.		
2	Pipet 2.5ml MilliQ water into the vial and mix.		
3	Transfer in aliquots of 100 µl to several 1.5ml tubes.		
4	Store the solution at 4ºC for one week or, preferably at -20ºC for 6 months.		
	Aprotinin, 10mg/ml		
1	Obtain a vial of 10mg aprotinin.		
2	Pipet 1ml MilliQ water into the vial and mix.		
3	Transfer aliquots of 100 µl to 1.5ml 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, 10mg/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 15ml conical tube with screw cap in a beaker on the pan of a balance capable of weighing mg quantities. Tare the balance.		

Batch Record for Downstream Processing of Anti-IL8 mAB: TFF Operation

	Solution	*Initials/Date*	*Verifier/ Date*
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 40mg 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: *Vol,ml* = mg PMSF ÷ 10mg/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 (but has a very short half-life in aqueous solutions).		

5.2 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.	



Batch Record for Downstream Processing of Anti-IL8 mAB: TFF Operation

#	*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 SOP).		
2	Remove the 4 plugs on the Pellicon XL (PXC030C50) cassette ports. Align the Pellicon XL device ports with Labscale 500ml 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.		

5.3 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 500ml 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 350ml 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 50ml 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.		

Batch Record for Downstream Processing of Anti-IL8 mAB: TFF Operation

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

5.4 Pre-conditioning the Pellicon filtration membrane

#	*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 50ml 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.		

5.5 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 head plate of the bioreactor, providing access to the cells and conditioned medium.		

Batch Record for Downstream Processing of Anti-IL8 mAB: TFF Operation

#	*Task*	*Initials/ Date*	*Verifier/ Date*
2	Transfer the culture to three 250ml centrifuge bottles using a 50ml pipet and PipetAid. Residual culture can be transferred to an Ehrlenmeyer flask for temporary storage.		
3	Centrifuge cells in pre-chilled Sorvall centrifuge, fitted with a SLA1500 rotor, at 1000 xg 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 on a 250ml Corning bottle. Apply the vacuum and complete filtration of the medium.		
6	Add protease inhibitors and Tween 80 as follows. To each 250ml bottle of CM supernatant, add 1ml 10mg/ml PMSF, 50 µl of 10mg/ml Aprotinin stock and 62.5 µl 2mg/ml Leupeptin stock. Also add 2.5ml 10% Tween 80 (final concentration will be near 0.1%).		

5.6 Concentration of Anti-Il8 Mab in conditioned medium

#	*Task*	*Initials/ Date*	*Verifier/ Date*
1	Remove the reservoir cover and fill the reservoir with anti-IL8 mAb sample (up to 500ml) to be concentrated.		
2	Ensure the vent port is open by removing the plug from the VENT port. Open the tank outlet valve.		
3	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.		
4	Adjust the retentate valve restriction by slowly turning the retentate valve clockwise until the retentate pressure gauge reads 10-psi.		
5	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.		
6	Concentrate the solution until the desired volume is reduced 10 fold or greater, but ideally down to about 20ml.		
7	Turn off the pump and empty the permeate container into a large bottle with a cap and label as: Anti-IL8 Mab, Permeate Waste, disposal; bleach then drain, [initials], [date].		
8	Measure the volume of the retentate in the reservoir		

Batch Record for Downstream Processing of Anti-IL8 mAB: TFF Operation

5.7 Buffer Exchange of the concentrated Anti-IL8 Mab conditioned Media

#	*Task*	*Initials/ Date*	*Verifier/ Date*
1	Add the Binding Buffer 20mM Phosphate Buffer pH 7.0 with 0.1% Tween 80 to the concentrated conditioned media to bring the volume back to the pre-concentrated volume		
3	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.		
4	Adjust the retentate valve restriction by slowly turning the retentate valve clockwise until the retentate pressure gauge reads 10-psi.		
5	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.		
6	Concentrate the solution until the desired volume is reduced 10 fold or greater, but ideally down to about 20ml.		
7	Turn off the pump and empty the permeate container into a large bottle with a cap and label as: Anti-IL8 Mab, Permeate Waste, disposal; bleach then drain, [initials], [date].		
8	Measure the volume of the retentate in the reservoir		
9	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.		

5.8 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 50ml conical tube).		
2	Disconnect the retentate tubing (silicone, translucent) from the retentate in port and open the retentate backpressure valve (turn counterclockwise). Fluid should now drain by gravity.		
3	When drainage ceases, rinse the Pellicon innards by injection of 5ml 20mM sodium phosphate buffer/0.1% Tween 80 from the retentate tube using a 10ml 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 to drain reservoir.		

Batch Record for Downstream Processing of Anti-IL8 mAB: TFF Operation

6	Stop the pump, close the outlet valve, and add 5ml 20mM sodium phosphate buffer/0.1% Tween80 to the chamber to rinse sides and effect collection of residual tPA. Pipet the solution along the walls repeatedly to rinse, then collect and transfer to the collection vessel.		
7	Reconnect the pump outlet tubing (Sta-Pure, white) to the Feed In port.		
8	Label the recovery collection vessel Concentrated tPA, [date], [initials].		
9	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.		

5.9 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 5.3)
2. cleaning with 0.1N NaOH.
3. flushing once more with MilliQ water (procedure 5.3).
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 500ml water, as described in procedure 5.3.		
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 500ml 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 250ml and then turn the pump off. Reconnect the retentate (silicone, translucent) tubing to the RET IN port.		

Batch Record for Downstream Processing of Anti-IL8 mAB: TFF Operation

#	*Task*	*Initials/ Date*	*Verifier/ Date*
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 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	Repeat Flushing with MilliQ water as described above in Procedure 5.3.		

5.10 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 10ml 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.		

5.11 Clean Base

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

6.0 Attchments

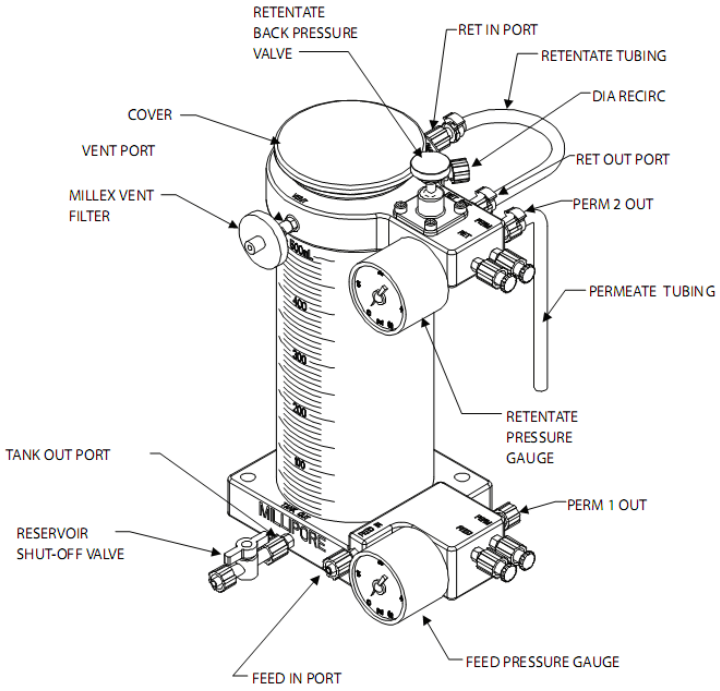
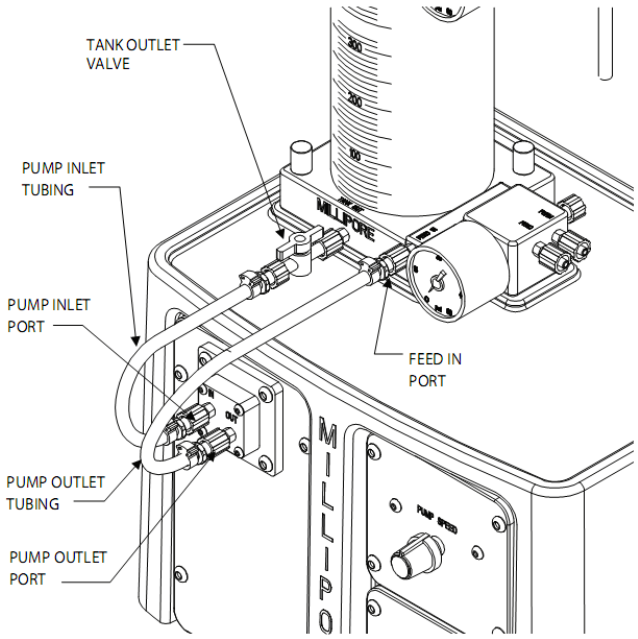


Figure 1: Reservoir Set Up
(http://www.millipore.com/userguides.nsf/docs/p60085)



Batch Record for Downstream Processing of Anti-IL8 mAB: TFF Operation

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

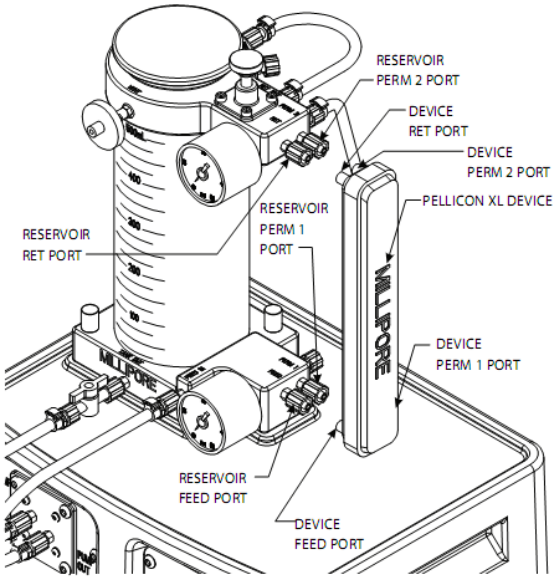


Figure 3: Installation of Pellicon XL Device
(http://www.millipore.com/userguides.nsf/docs/p60085)

7. 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.