Batch Record for Downstream Processing of cNIST mAb
Lot Number: _____

Approvals:
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1.0 Harvest, Centrifugation, and Concentration

1.1 Description

This batch record directs and documents the isolation of nist 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 cNIST mAB in conditioned growth medium by tangential flow filtration

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

1.2 References

Title	*Doc #*
SOP: End-of-Run cNIST 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			

1.4. Materials

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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. Reagents

Reagent Name	*Vendor Name*	*Catalog #*	*Initials/Date*	*Verifier/ Date*
Sodium Hydroxide (NaOH)				
Tween 80				
1X PBS				
MilliQ Water				
Halt cocktail protease inhibitor solution (100X)				

1.6 Procedure:

1.6.1. Preparation of Solutions

	Solution	*Initials/Date*	*Verifier/ Date*
Step	**0.1N NaOH for cleaning**		
1	Weigh 3.2 g ± 0.05g NaOH		
2	Transfer the solid NaOH to a 1000 ml beaker with stir bar.		
3	Measure 700ml of milliQ water with a 1000ml measuring cylinder and add to the beaker containing NaOH		
4	Add magnetic stir bar and stir the solution to dissolve the NaOH solids by placing the beaker on the stirrer plate		
5	When the NaOH is completely dissolved, bring the solution to its final volume of 700ml with milliQ water		
6	Sterile filtered the solution with 0.2 micron vacuum filter. Label the bottle: 0.1N NaOH, Date, Initials, Team Name		
7	Store the bottle at Room Temperature		

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	0.05N NaOH for Pelicon XL cassette storage	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.		
3	Sterile filter the prepared solution with 0.2-micron syringe filter attached to a 10ml syringe in a sterile 15ml conical tube. Label the tube appropriately		
	10% w/v Tween 80	Initials/Date	Verifier/ Date
1	Measure 80 ml MilliQ water using a 100ml measuring cylinder and transfer to a 200ml clean beaker. Add 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 solution (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	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	Sterile filter the prepared solution with 0.2 micron vacuum filter ssytem		
8	Store the solution in an appropriately labeled bottle at room temperature.		
	1X PBS with 0.1% Tween 80	Initials/Date	Verifier/ Date
1	Measure 79.2 ml of 1X PBS with 100 ml graduated cylinder		
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		

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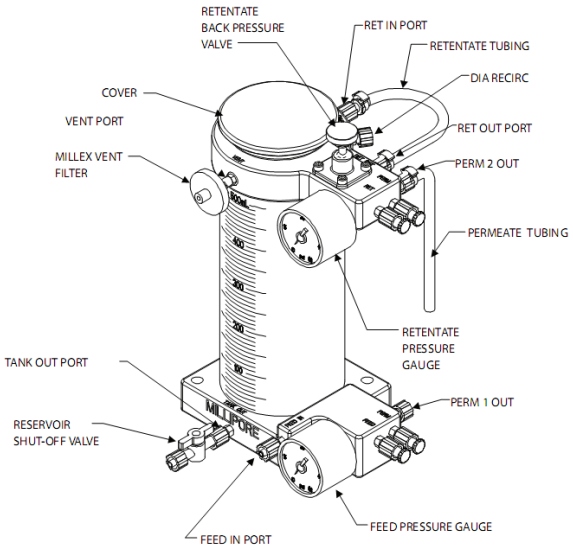
6	Store the solution at room temperature		
	Sterile filtered MilliQ water	Initials/Date	Verifier/ Date
1	Measure 1000ml milliQ water and sterile filter the water using a 0.2-micron vacuum filter and labelled the bottle as sterile filtered milliQ water, date and initials		

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 Reservoir ports being sure the PERM and RET DEVICE ports of		

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	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.		
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.		

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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 culture 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 100ml 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 2500x *g* for 10 min, 4 degrees C.Repeat with the rest of the culture medium		
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.		
5	Add protease inhibitors and Tween 80 as follows:		

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	To each 250 ml of Conditioned medium, add 250µl of 100X halt protease inhibitor cocktail stock to achieve 1X final concentration. Also add 2.5 ml 10% Tween 80 (final concentration will be near 0.1%).		

1.6.6.Concentration of Anti-Il8 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 cNIST mAB sample (up to 500 ml) to be concentrated.		
3	Ensure the 0.2µm syringe filter is attached to the VENT port.		
4	Open the tank outlet valve		
5	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.		
6	Adjust the retentate valve restriction (black knob on top) by slowly turning the retentate valve clockwise until the retentate pressure gauge reads 10 psi.		
7	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.		
8	Concentrate the solution until approximately 40ml of retentate is left		
9	Turn off the pump and empty the permeate container into a large bottle with a cap and label as: cNIST Mab, Permeate Waste, disposal; bleach then drain, [initials], [date].		
10	Measure the volume of the retentate in the reservoir with the 50 ml serological pipette Volume of the retentate in reservoir: _____ml		

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 into the 50 ml conical tube		

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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	Turn pump dial to stop the pump. Reconnect the pump outlet tubing (Sta-Pure, white) to the Feed In port.		
7	Close the tank outlet valve		
8	Add 10 ml of 1X PBS with 0.1% Tween 80 to the reservoir. Open the tank outlet valve		
9	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.		
10	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.		
11	Recirculate the 1X PBS with 0.1% Tween80 for 10 minutes and then turn the pump off.		
12	Disconnect the pump outlet tubing (Sta-Pure, white) from pump outlet port and place in cleaned sterile 50 ml conical tube used in step 1 of 1.6.7. Disconnect the male luer end of the permeate tubing from recirculation port and place it in waste collection vessel.		
13	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.		
14	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		
15	Replace retentate tubing (silicone, translucent) in retentate port. Reconnect pump outlet tubing (Sta-Pure, white).		
16	Disconnect FEED IN tubing and place in collection vessel. Open tank outlet valve, turn pump speed up and let the reservoir drain.		

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17	Stop the pump, close the outlet valve and reconnect the pump outlet tubing (Sta-Pure, white) to the Feed In port		
18	Label the recovery collection vessel as Concentrated cNIST mAb, [date], [initials], company name. Measure and record the volume. Volume of concentrated cNIST mAb: _____ml		
19	Store for the short term (1 week) in 2°C-8°C refrigerator for use in further purification steps.		

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		

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	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. Open the retentate valve by turning it anti-clockwise		
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*		

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

1.6.11. Attchments

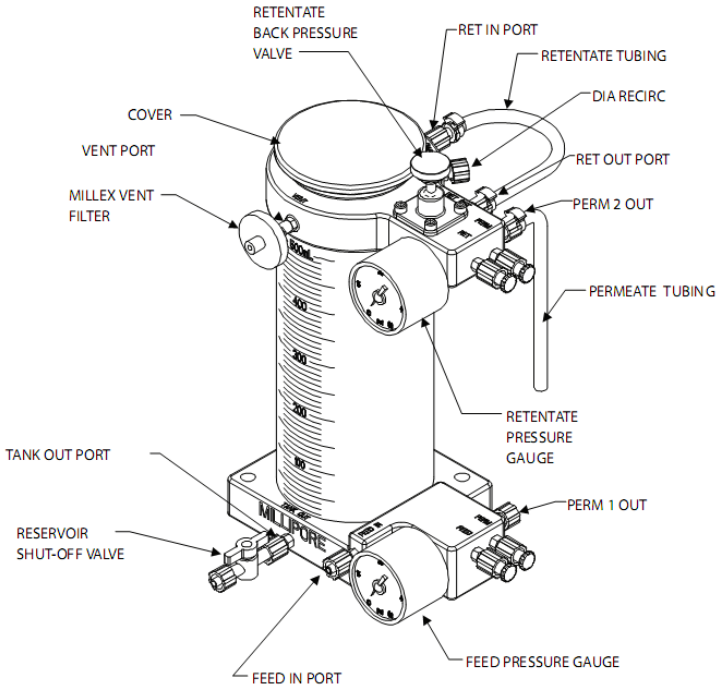

Figure 1: Reservoir Set Up

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

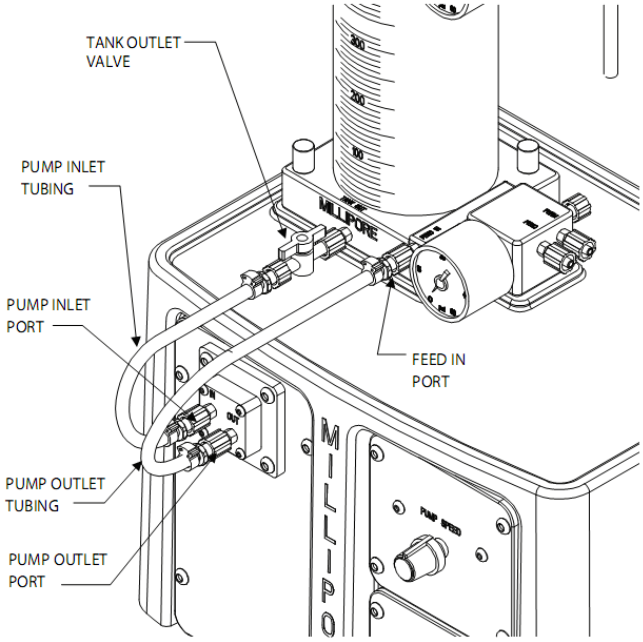

Figure 2: Pump Base Set Up
(http://www.millipore.com/userguides.nsf/docs/p60085)

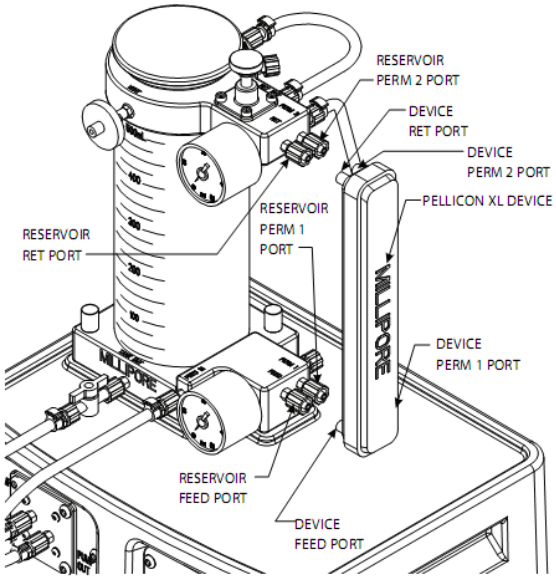

Figure 3: Installation of Pellicon XL Device
(http://www.millipore.com/userguides.nsf/docs/p60085)

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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 (cNIST) from Conditioned Medium by Protein A Affinity Chromatography on the ÄKTApure Chromatography System	DP012
SOP: Operation of AKTA pure Chromatography System	DP 5
SOP: Operation of NanoDrop 2000 Spectrophotometer	QCB 11

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			

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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 3.2 ± 0.02g $Na_2HPO_4.7H_2O$ 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		
4	Transfer the solution to a 250 ml graduated cylinder. Adjust the final volume to 200 ml		

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5	Sterile 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	Sterile filter the solution with a 0.22 µm filter. Degassing is not necessary.		

2.6.2. Start-up and preparation of AKTA Chromatography Instrument and Computer

#	*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	The On/Off switch for the instrument is located on the right side toward the rear of the housing. Switch to the 'On' position		
4	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		
5	Login to the computer using credentials provided by the College		

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6	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.		
7	Open the System Control window (under Tools menu, if not opened automatically on startup)		
8	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		

2.6.3. Priming the pump rinsing system

#	*Task*	*Initials/Date*	*Verified Initial/Date*
1	Remove the pump rinsing liquid tube from the holder located on the right-hand bottom corner of the system		
2	Fill the pump rinsing liquid tube with 50ml of 20% ethanol		
3	Place the pump rinsing liquid tube back in the holder		
4	Insert the inlet tubing to the system pump piston rinsing system in the rinsing solution tube.		
5	Connect a 25 to 30 ml syringe to the outlet tubing of the system pump piston rinsing system. Draw liquid slowly into the syringe		
6	Disconnect the syringe and discard its contents.		
7	Fill the rinsing solution tube so that the tube contains 50 ml 0f 20% ethanol.		

2.6.4 Prime inlets and purge pump heads

#	*Task*	*Initials/Date*	*Verified Initial/Date*
1	Make sure that all inlet tubing that is to be used during the method run is placed in the correct buffer		
2	Turn on the AKTA pure system if not already on		
3	Open the unicorn 6.3 software		
4	Open the **system control** module in the unicorn 6.3 software		
5	In the **Process Picture** click on the buffer inlets and select B2		

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6	Connect 20 ml syringe to right pump head of pump system B Make sure that the syringe fits tightly		
7	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.		
8	Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents		
9	In the Process Picture click on the buffer inlets and select B1		
10	Connect 20 ml syringe to left pump head of pump system B Make sure that the syringe fits tightly		
11	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		
12	Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents		
13	In the Process Picture click on the buffer inlets and select A2		
14	Connect 20 ml syringe to right pump head of pump system A Make sure that the syringe fits tightly		
15	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		
16	Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents		
17	In the Process Picture click on the buffer inlets and select A1		
18	Connect 20 ml syringe to left pump head of pump system A Make sure that the syringe fits tightly		
19	Connect 20 ml syringe to right pump head of pump system A Make sure that the syringe fits tightly		
20	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		
21	Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents		
22	Verify that the piece of waste tubing connected to the injection valve port W1 is placed in a waste vessel		

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23	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		
24	In the **Process Picture** click on the pumps		
25	Set **Conc % B** to 100% B and click **Set % B**. only system pump B is active		
26	In the **Process Picture** click on the buffer inlets and select B1. The inlet valve switches to the selected port.		
27	In the Process Picture click on the pumps		
28	Set the **System flow** to 1.0 ml/min. Click **Set flow rate**.		
29	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		
30	Open the purge valve by turning it counterclockwise about three quarters of a turn. Draw a 5ml of liquid slowly into syringe.		
31	Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.		
32	Connect the syringe to the purge valve on the right pump head of System pump B, Make sure that the syringe fits tightly into the purge connector		
33	Open the purge valve by turning it counterclockwise about three quarters of a turn. Draw a 5ml of liquid slowly into syringe		
34	Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents. Keep the system flow running		
35	To validate purge of pump B,In the **Process Picture** click on the **Injection valve** and select **Manual Load**.		
36	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.		
37	In the **Process Picture** click on the **injection valve** and select **System pump waste.** The injection valve switches to waste position.		
38	In the **Process Picture** click on the pumps		
39	Set **Conc % B** to 0% B and click **Set % B.** only system pump A is active		
40	In the **Process Picture** click on the buffer inlets and select A1		

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41	Set the **System flow** to 1.0 ml/min. Click **Set flow rate**. A system flow starts		
42	Connect a 20 ml syringe to the purge valve of the left pump head of system pump A. Make sure that the syringe fits tightly into the purge connector.		
43	Open the purge valve by turning it counterclockwise about three quarters of a turn. Draw a 5ml of liquid slowly into syringe.		
44	Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.		
45	Connect the syringe to the purge valve on the right pump head of System pump A. Make sure that the syringe fits tightly into the purge connector		
46	Open the purge valve by turning it counterclockwise about three quarters of a turn. Draw a 5ml of liquid slowly into syringe		
47	Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.		
48	To validate purge of pump A, In the **Process Picture** click on the **Injection valve** and select **Manual Load**		
49	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.		
50	Confirm that the HiTrap protein A HP 1ml column is installed on a chromatography system. If not refer to "SOP: Isolation of mAb (anti IL-8) from Conditioned Media by Protein A Affinity Chromatography on the ÄKTApure System" Document # DP12		

2.6.5. Preparation and Installation of 10 ml SuperLoop

#	*Task*	*Initials/Date*	*Verified Initial/Date*
1	Rinse and dry the entire disassembled Superloop parts if not done already.		
2	Insert the 18 cm tube into a female threaded connector (black).		
3	Connect the male threaded connector to the female threaded connector inserted into an 18 cm tubing. Make sure the tube is snug tight		
4	Connect the male threaded connector to one of the inner end pieces.		

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5	Insert the 28 cm tube into a female threaded connector (black)		
6	Connect the male threaded connector to the female threaded connector inserted into a 28 cm tubing. Make sure the tube is snug tight		
7	Rinse/wet O-rings on the end pieces and movable seal with deionized water.		
8	Insert the movable seal into the graduated glass tube from the bottom (zero) end in such a way that the end with O-ring is closest to 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	Mount the glass tube on a lab stand with clamp. Working over a sink or container to catch any overfill, pipet enough buffer A into the upper portion of the tube to fill it.		
10	Mind the liquid that will squirt from the tubing; direct it into the sink. Insert the inner end pieces with the 18 cm tubing attached into the glass tube, contacting the liquid meniscus to eliminate air bubble entrapment. Press the end piece completely into the glass tube		
11	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.		
12	Rotate the bottom inner end piece with 28 cm tubing 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.		
13	Remove the glass tube with end pieces from the clamp.		
14	Attach the bottom outer end piece by threading it onto the glass tube.		
15	Slide the plastic protective jacket over the glass tube and seat it firmly into the bottom outer end piece.		
16	Attach the top outer end piece to the remaining exposed threaded end of the glass tube		

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17	To install the assembled Superloop 10 onto the AKTA 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		
18	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.		
19	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'.		
20	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.		
21	Confirm the inlet tubing A1 is in the Buffer A container		
22	Using the manual control panel in the System Control window, click on Pump A in the diagram and select "Pump A Wash"		
23	Upon completion of the wash, set the flow rate to 1 ml/min. Click on the injection valve depiction and select "Inject".		
24	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.		
25	Fill a 10 ml syringe with buffer A and inject sufficient volume to completely fill the Superloop.		
26	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.		
27	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.		

2.6.6. pH Electrode Calibration

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Task	*Initials/Date*	*Verified Initial/Date*
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.		
In the Unicorn System Control window, choose 'Calibration' from the System menu. From the drop down menu under 'Monitor to calibrate', select 'pH'.		
Click the 'Prepare for Calibration' button. You will hear the valve switch to the calibrate position.		
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		
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.		
Thoroughly rinse the syringe with 3-4 changes of MilliQ water. Wash the pH flow cell by injecting water into pH valve port **Cal**.		
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.		
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.		

2.6.7. Preparation of fraction collector

#	*Task*	*Initials/Date*	*Verified Initial/Date*
1	Prepare 30 fraction collector tube by adding 200µl 1M Tris pH 9.00 to the bottom of each tube		
2	Load prepared 30 collection tubes into the fraction collector starting at position 1.		
3	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		

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	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.		
4	Gently raise the arm and swing it into position against tube 1		

2.6.7. 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 start 4) Allow the method to run to completion (about 15 minutes).		

2.6.8. Protein A Affinity Chromatography

Chromatographic run sequence summary:

1) Inject 5.0 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.

#	*Task*	*Initials/Date*	*Verified Initial/Date*
1	Obtain the concentrated cNIST mAb with your company name (prepared in step 1.6.7.)		

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	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 5.5ml of filtered sample, being careful to avoid or eliminate any air bubbles 2) Dispense excess sample back into its original container, retaining 5.5 ml in the syringe 3) Insert syringe firmly into sample inlet port with Luer lock tightened 4) Inject 5.5 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_5ml* 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		

2.6.9. 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.		

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	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.		

2.6.10. Equipment shut-down and short term (less than 3 days) storage

#	*Task*	*Initials/Date*	*Verified Initial/Date*
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).		
2	In the Unicorn software, open the System Control window.		
3	Under the File menu, choose Open, then select the method 'System Short Term Storage'		
4	Click Start		
5	Allow the method to run to completion, as indicated by an audible tone and onscreen window.		

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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 into the calibration port. Leave the syringe attached.		
7	Turn off the instrument or perform the long-term storage routine as needed		

2.6.11. Cleaning of the Superloop 10 sample holder- short term.

#	*Task*	*Initials/Date*	*Verified Initial/Date*
1	For short term storage of the Superloop on the AKTA instrument, inject 2 ml Milli-Q water into the sample chamber		
2	Pump it out to waste by temporarily disconnecting the outlet tubing that is connected to the injection valve at port 'loop F'.		
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		
4	Reconnect to 'loop F'		
5	Repeat steps 1 through 4 on this section three times		
6	Inject 10 ml water into the sample chamber of the Superloop.		

2.6.12. Equipment shut-down and long term (3 days or more) storage

#	*Task*	*Initials/Date*	*Verified Initial/Date*
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).		
2	In the Unicorn software, open the System Control window.		
3	Confirm that the pH valve is in the 'Bypass' or 'Restrictor' position		
4	Under the File menu, choose Open, then select the method 'System Long Term Storage'.		
5	Click Start		
6	Allow the method to run to completion, as indicated by an audible tone and onscreen window.		

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7	Turn off the instrument.		
8	Remove the Superloop from the instrument and carefully disassemble it. Hand wash all parts with a general purpose lab cleaner, rinse well and allow to air dry. Store the dried components in their original box		

3.0 History

Revision Number	***Effective Date***	***Preparer***	***Description of Change***
0	17JAN24	Hetal Doshi	Initial release