Mount Wachusett Community College
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Batch Record: Downstream Process Isolation of Anti-IL8 mAb from CHO Cells: Chromatography Operation

1.0 Description

1.1 This batch record covers the precise operating steps necessary to purify recombinant mAb from conditioned cell culture medium, including harvest, clarification, ultrafiltration and protein A affinity chromatography with the BioRad Biologic LP Chromatography instrument.

2.0 Reference

Title	*Document Number*
SOP: Isolation of anti IL-8 mAb by Protein A Affinity Chromatography on the Biologic LP Chromatography System	
Biologic LP Chromatography Equipment Operation SOP	

3.0 Equipment

Equipment Type	*Manufacturer, Model Number*	*Calibration Due Date*	*Initials/Date*	*Verifier/Date*
Chromatography System	BioRad Biologic LP Chromatography System			
Column	BioRad Bio-Scale Mini Affi-Prep Protein A column, 1ml ***Note: remove the column from 4ºC storage and allow to come to room temperature***	N/A		

4.0 Components

Component	*Quantity Required*	*Quantity Used*	*Initials/Date*	*Verifier/Date*
Fraction tubes	20			
Syringe, 10 ml	1			
Ehrlenmeyer flask, 125 ml	1			
Ehrlenmeyer flask, 500 ml	1			

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5.0 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 citric acid, pH 3.0		200 ml		
Deionized water	Filtered, degassed deionized water		500 ml		
System Storage Solution	20% Ethanol		300 ml		
Neutralizer	1 M Tris base		100 ml		

6.0 Procedure

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 0.80 ± 0.02 gm NaH_2PO_4 and transfer to a 1 L flask with magnetic stir bar.		
2	Weigh 3.60 ± 0.02 gm $Na_2HPO_4 \cdot 7H_2O$ and transfer to the same flask.		
3	Measure 980 ml deionized water in a graduated cylinder and add the water to the solids in the flask..		
4	Stir until the solids have dissolved, then adjust the pH to 7.0.		
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 citric acid, pH 3.0		
1	Weigh 3.84 gm citric acid in a 400 ml beaker with magnetic stir bar.		
2	Dissolve in 180 ml deionized 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		
5	Filter the solution, allowing it to degas for 15 – 20 minutes. Label appropriately		

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	1M Tris base: neutralizer.		
1	Weigh 12.11 gm Tris base [tris(hydroxymethyl)aminomethane] into a plastic weigh boat and transfer to a 200 ml beaker with a stir bar.		
2	Measure 85 ml deionized water in a graduated cylinder and transfer the water to the beaker containing Tris powder. Stir until dissolved.		
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.		

6.2 Clarification and Ultrafiltration of Conditioned Medium

#	*Task*	*Initials/Date*	*Verifier/Date*
1	Transfer conditioned medium to centrifuge bottles; centrifuge at 1000 x g for 10 minutes at 4ºC.		
2	Decant the supernatant into a beaker (for smaller volumes that are to be filtered with a syringe-mounted filter) or directly into a bottle top 0.2µm filter. Filter the supernatant into a 50 ml conical tube or bottle.		
3	Transfer 15 ml of the filtered supernatant into each of two Centricon 15 (30kDa) ultrafiltration devices. Centrifuge in the table top centrifuge with swinging bucket rotor at 3500 x *g* , 4ºC for 25 minutes.		
4	Remove the filter insert, pour off the filtrate in the bottom tube and return the insert to the tube. Add additional CM to fill the upper chamber and repeat centrifugation.		
5	Repeat step 6.2.4 until sufficient quantity (50-75 ml of starting material) of the supernatant has been concentrated.		
6	Resuspend the concentrate in buffer A and repeat centrifugation.		
7	Remove the concentrate with a 200µl or 1000µl pipettor and place in a small beaker. Rinse the ultrafiltration membrane multiple times with 1 ml aliquots of buffer A (at room temperature) and combine the rinses with the		

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	concentrate in the beaker, until a volume of approximately 10 ml has been reached.		
8	Mix the concentrate and rinses, then draw into a 10 ml syringe. Mount a 0.22µm filter on the syringe and pass the mAb-containing solution through it into a clean beaker.		
	***Note**: The sample must be at room temperature prior to application to the column.*		

6.3 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 Buffer A 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 Buffer B to the bottom of the container. Approximate volume of Buffer B:_____ ml		
3	Verify that the Waste effluent tubing is placed in a 500 mL E flask		
4	Place an adequate supply of tubes (30), numbered sequentially, in the fraction collector carousel.		
5	Pipet 200 µl 1M Tris into each tube in the carousel; ensure that the aliquot gets to the bottom of the tube (as opposed to clinging to the side).		
6	Rotate the tube carousel so that the #1 position is set to receive the initial drops.		
7	Turn the Biologic LP system on. The on/off switch is on the lower left side on the front of the instrument.		
8	Turn on the computer and login		
9	Open the LP Data View software		
10	Confirm that the installed column is a BioRad Bio-Scale Mini Affi-Prep Protein A column, 1ml		

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6.4 Protein A Affinity Chromatography

Chromatographic run sequence is as follows:

1) Inject 9.5 ml from the 15 ml Falcon tube as Buffer C; 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	Record the sample information. Sample origin: Batch #: Date prepared: Volume: pH:		
2	Initiate the run: 1.1.1. 1) Press the List of Methods softkey, select the method with file name “Anti IL8”. 1.1.2. Press the Run mode softkey. 1.1.3. Press Record on the LP Data Veiw software. Observe that the fraction collector is receiving drops		
3	Upon completion, transfer the labeled tubes to a tube rack and store at 4ºC for later analysis.		

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6.7 ***Evaluate Chromatographic Separation***

#	*Task*	*Initials/Date*	*Verified Initial/Date*
1	Print the chromatogram or save to a USB drive.		
2	*Optional.* Determine protein content per fraction by Bradford Protein Estimation. Refer to the SOP for that procedure.		