Downstream Process Batch Record: 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 AKTA pure instrument.

2.0 Reference

Title	*Document Number*
SOP: Isolation of mAb (anti IL-8) from Conditioned Medium by Protein A Affinity Chromatography on the ÄKTApure Chromatography System	
AKTApure 25 Equipment SOP	DP xx
SOP: Bradford Protein Assay	
SOP: Determination of Antibody Titer in Recombinant mAb Preparations	QCB

3.0 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		

4.0 Components

Component	*Quantity Required*	*Quantity Used*	*Initials/Date*	*Verifier/Date*
Fraction tubes	30			
Syringe, 10ml	1			
Ehrlenmeyer flask, 125ml	1			
Ehrlenmeyer flask, 500ml	1			

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5.0 Solutions

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

6.0 Procedure

6.1 Preparation of Buffers and Solutions			
#	*Task*	*Initials/Date*	*Verifier/Date*
	<u>Buffer A: Binding buffer</u>: 20mM 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 $Na_2HPO_4 \bullet 7H_2O$ 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 400ml beaker with magnetic stir bar.		
2	Dissolve in 180ml MilliQ water.		
3	Adjust the pH dropwise with 10N NaOH, to a final pH of 3.0		
4	Transfer the solution to a 250ml graduated cylinder. Adjust the final volume to 200ml		

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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 200ml beaker with a stir bar.		
2	Measure 90ml 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 100mlgraduated cylinder, rinsing the beaker with small aliquots of water, which are then added to the cylinder until a final volume of 100ml is obtained.		
4	Filter the solution with a 0.22 µm filter. Degassing is not necessary.		

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 125ml E. flask		
4	Verify that the Waste effluent tubing labeled W, W1, and W2, are placed in a 500ml E flask		
5	Place an adequate supply of tubes (30), numbered sequentially, in the fraction collector carousel.		

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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 1ml (at room temperature).		

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 10ml 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 10ml 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 10ml of the second pH standard buffer. Connect the syringe to		

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	the Luer connector of pH valve port **Cal**, and inject the 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.		

6.4 Column conditioning

#	*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*HiTrap Protein A-HP 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.		

6.6 Protein A Affinity Chromatography

Chromatographic run sequence summary:
1) Inject 9.5ml from the Superloop; begin collecting 5ml fractions; flow rate = 0.5ml/min.
2) Wash unbound proteins through with up to 15-column volumes (CV) buffer A, until A280 stabilizes; collecting 2.5ml fractions. Flow rate = 1ml/min.
3) Elute bound immunoglobulins with step to 0.1M Na-citrate, pH 3; collecting 1ml 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.

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#	*Task*	*Initials/Date*	*Verified Initial/Date*
1	Obtain the concentrated Anti-IL8 mAb sample in the Buffer A collected from TFF. Sterile filter the sample using 10ml syringe and 0.22 µm syringe filter in a 50ml conical tube. Record the sample information Sample origin: Batch #: Date prepared: Volume: pH:		
2	Sample injection into 10ml Superloop: 1) Fill 10ml 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 10ml 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* 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		

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6.7 ***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.		