Batch Process Record: tPA Production from CHO Cells: AKTA pure Chromatography Operation

Approvals

Preparer: Dr. David Frank Date: 20APR16
Reviewer: Jason McMillan Date: 21APR16
Reviewer: Dr. Maggie Bryans Date: 21APR16

1.0 Description

1.1 This batch production record covers the precise operating steps necessary to purify recombinant tissue-type plasminogen activator from concentrated conditioned cell culture medium using cation exchange chromatography.

2.0 Reference

Title	Document Number
Batch Record for Downstream Processing of t-PA: TFF Operation	
End-of-Run t-PA Process SOP: Harvest, Centrifugation, Concentration, pH Adjustment	DP 3
AKTA pure 25 Equipment SOP	DP 5
SOP: Bradford Protein Assay	
SOP : tPA ELISA	QCB 1

3.0 Equipment

Equipment Type	*Manufacturer, Model Number*	*Calibration Due Date*	*Initials/Date*	*Verifier/Date*
Chromatography System	GE Healthcare AKTA pure 25			
Column	HiTrap SP, 5ml	N/A		

4.0 Components

Component	*Quantity Required*	*Quantity Used*	*Initials/Date*	*Verifier/Date*
Fraction tubes	35			
Syringe, 10 ml	1			
Erlenmeyer flask, 125 ml	1			
Erlenmeyer flask, 500 ml	1			

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5.0 Solutions

Solution	*ID*	*Date Prepared*	*Volume Required*	*Volume Used*	*Initials/ Date*
Buffer A	0.2M NaOAc, pH 5.0, 0.1% Tween 80		500 ml		
Buffer B	0.2M NaOAc, pH 5.0, 0.1% Tween 80, 1M NaCl		500 ml		
Filtered, degassed MilliQ water			500 ml		
20% Ethanol			500 ml		

6.0 Procedure

6.1 Preparation of Buffers and Solutions			
Buffers A & B: 0.2M Sodium Acetate, pH 5.0, 0.1% Tween 80 +/- 1M NaCl			
#	*Task*	*Initials/Date*	*Verifier/Date*
1	Dissolve 11.5 ml glacial Acetic Acid (HOAc) in 950 ml filtered deionized water in a one liter beaker, with stir bar.		
2	Prepare 10M NaOH by dissolving 40.0 gm solid in filtered deionized water. Adjust the final volume to 100 ml. Use CAUTION as the solution becomes very hot, and is caustic.		
3	Titrate the pH of the HOAc solution to 5.0 by addition of 10M NaOH. Use a 3 ml transfer pipet to add about 15 ml to the HOAc, then proceed dropwise until pH 5.0 is obtained.		
4	Add 10 ml of 10% Tween 80, then adjust the final volume to 1000 ml. Set aside 500 ml of this solution and label ‘Buffer A’ along with its precise composition and date of preparation. Filter and degas the buffer by passage through a vacuum filter device attached to house vacuum, leaving the filtered solution under vacuum for 15-20 minutes.		

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5	Use the remaining 500 ml to dissolve 29.22 gm of NaCl in a 600 ml beaker. Following dissolution, filter and degas this solution and label the bottle 'Buffer B', along with the actual contents (0.2 M NaOAc, pH 5.0, 0.1% Tween80, 1 M NaCl).		

6.2 Chromatography system setup

#	*Task*	*Performed Initial/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	Confirm that an adequate supply (35) of tubes are placed in the fraction collector carousel.		
6	Rotate the tube carousel so that the #1 position is set to receive the initial drops. Lift the arm		

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	and swing it over to rest against the side of the first tube.		
7	Turn the AKTA pure system on. The on/off switch is on the right side toward the rear of the instrument.		
8	Turn on the computer and login		
9	Open the Unicorn 6.3 software by: 1) double clicking the desktop icon 2) clicking 'OK' at the Log On-Unicorn dialog box		
10	Confirm that the installed column is a HiTrap SP HP 5 ml		

6.1 Column conditioning

#	*Task*	*Performed Initial/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 SP 5ml 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.		

6.2 tPA Chromatography

#	*Task*	*Performed Initial/Date*	*Verified Initial/Date*
1	Record the sample information. Sample origin:		

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	Batch #: Date prepared: Volume: pH:		
2	Using a 0.2 µm filter mounted to a 30 ml syringe, filter the sample (concentrate from TFF, adjusted to pH 5) into a 50 ml tube.		
3	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		
4	Initiate the run: 1) Using the Unicorn 6.3 software, open the System Control window. 2)Under the File menu, choose Open*HiTrapSP tPA Production* 3) In the resulting dialog box, input Sample Info into the designated cell. 4) Enter 5) Click Next until the Start button is shown in the dialog box. 6) Click Start to begin the separation process.		

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Chromatographic run sequence is as follows:

1) Inject entire contents of 10 ml Superloop; begin collecting 5 ml fractions.
2) Wash unbound proteins through with up to 12 column volumes (CV) buffer A, until A280 stabilizes; eluent directed to waste.
3) Elute bound protein (mostly contaminants, with minor amount of t-PA) with linear gradient of 0-0.2M NaCl in 5CV; collect 5 ml fractions.
4) Hold at 0.2M NaCl for 2CV.
5) Elute bound t-PA with 0.5M NaCl in 2 CV; collect 2 ml fractions.
6) Flush column with 2 CV of 1.0M NaCl; collect 5 ml fractions.
7) Re-equilibrate column in 5 CV buffer A.

6.3 Evaluate Chromatographic Separation

#	*Task*	*Performed Initial/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	Use Operations\Fraction Histogram to indicate average protein content per fraction.		
5	Use Operations\Activity Histogram to enter amount of tPA per fraction, as determined using the tPA ELISA; assay dilutions of 1:1000, 1:10,000 for most fractions and the sample applied to the column; assay		

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	dilutions of 1:1000, 1:10,000 and 1:100,000 □l from fractionseluted with 0.5 M NaCl; refer to the tPA ELISA SOP.		
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. tPA from TFF on HiTrap SP 09APR15) 5) Print a copy of the chromatogram for record keeping		
7	Save changes.		

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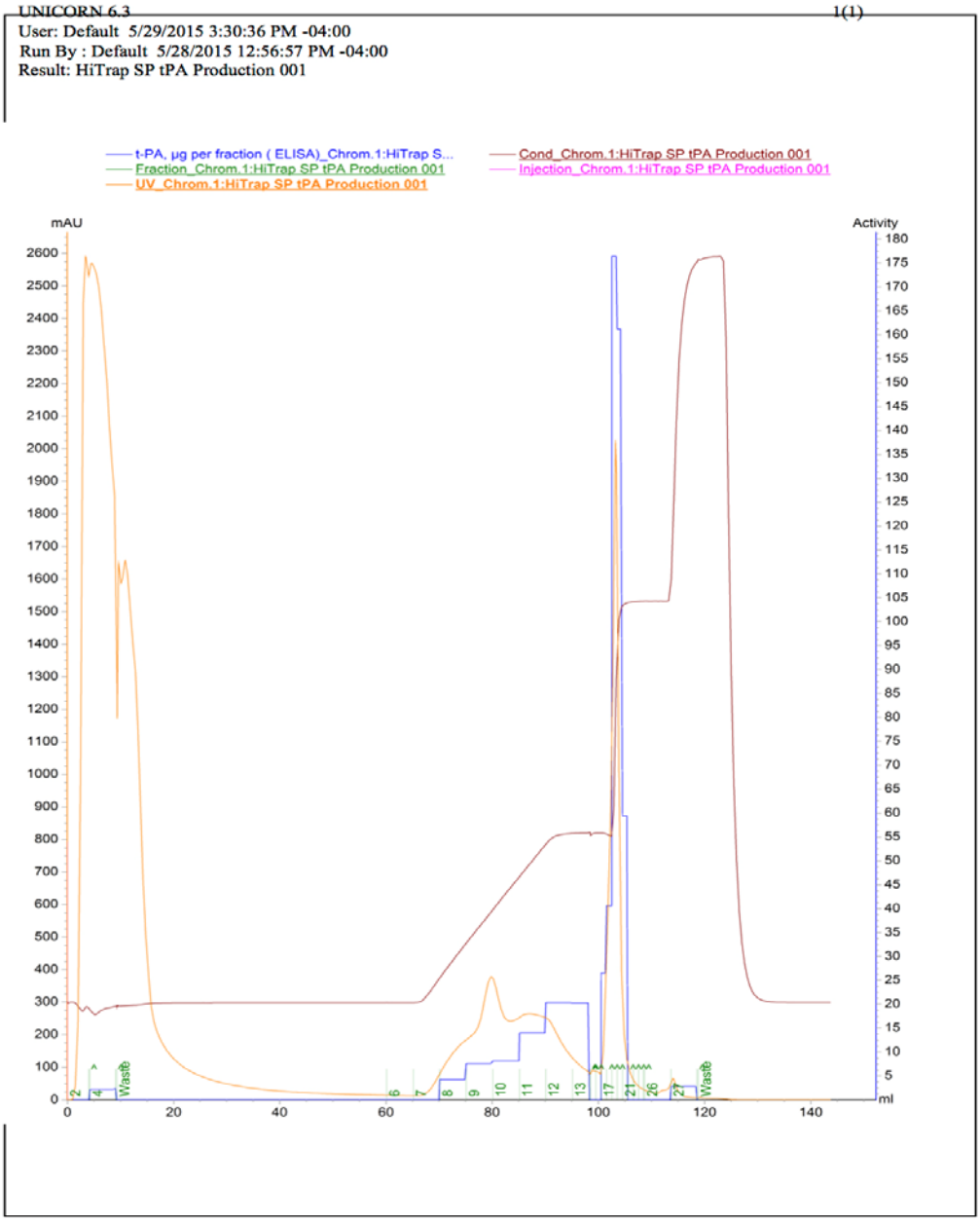

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Attachment:
Figure 1. Sample Chromatogram