

Title: Ion Exchange Chromatography of tPA SOP

Approvals:

Preparer: _____ Kari Britt _____ Date _____ 02Jun09 _____
Reviewer: _____ Bob O'Brien _____ Date _____ 02Jun09 _____

1. Purpose:

1.1. To purify tPA using ion exchange chromatography.

2. Scope:

2.1. Applies to purifying tPA using POROS 50HS resin and the BioLogic LP system.

3. Responsibilities:

- 3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
- 3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

4. References:

- 4.1. POROS 50HS Manufacturer's Instructions.
- 4.2. pH meter SOP
- 4.3. Amicon/Millipore column assembly SOP
- 4.4. BioLogic LP SOP

5. Definitions:

- 5.1. CV: Column Volume; $CV = \pi(L \text{ in cm})[(\text{radius of column in cm})^2]$
- 5.2. L = Length of column (meaning the height of the bead bed)
- 5.3. HETP: Height Equivalent to Theoretical Plate; $HETP = L/N$
- 5.4. $N = 5.54 (t_R/w_{1/2})^2$
- 5.5. t_R : retention time
- 5.6. $w_{1/2}$: peak width at half height
- 5.7. h: Reduced Plate Height; $h = HETP/D_p$
- 5.8. D_p : bead diameter

6. Precautions:

- 6.1. 0.1M NaOH is very corrosive. It is extremely damaging to eyes and mucous membranes. It causes burns. Avoid contact with skin. Harmful if swallowed or inhaled.

7. Materials:

- 7.1. Amicon Vantage-L Biochromatography column and accessories
- 7.2. POROS 50 HS Cation exchange packing medium (2-8°C)
- 7.3. BioRad BioLogic LP System
- 7.4. 0.22 μm sterile filter units
- 7.5. pH paper
- 7.6. waste beakers
- 7.7. laboratory film, such as, Parafilm
- 7.8. ring stand with clamps
- 7.9. 1mL syringe
- 7.10. MilliQ water
- 7.11. Equilibration Buffer A: 20mM phosphate buffer pH 6
- 7.11.1. NaH_2PO_4 (sodium phosphate monobasic, anhydrous)

Title: Ion Exchange Chromatography of tPA SOP

- 7.11.2. Na₂HPO₄·7H₂O (sodium phosphate dibasic, heptahydrate)
- 7.12. Elution Buffer B: 20mM phosphate buffer pH6, 1M NaCl
 - 7.12.1. NaH₂PO₄ (sodium phosphate monobasic, anhydrous)
 - 7.12.2. Na₂HPO₄·7H₂O (sodium phosphate dibasic, heptahydrate)
 - 7.12.3. NaCl (sodium chloride)
- 7.13. Cleaning Solution 0.1M NaOH

8. Procedure:

8.1. Prepare buffers and solutions

8.1.1. Buffer A: Equilibration Buffer, 20mM Phosphate, pH 6

- 8.1.1.1. Weigh 2.10 ±0.05 grams of sodium phosphate monobasic anhydrous.
- 8.1.1.2. Weigh 0.66 ±0.02 grams of sodium phosphate dibasic heptahydrate.
- 8.1.1.3. Transfer both chemicals to a 1L vessel.
- 8.1.1.4. Using a 1L graduated cylinder, measure approximately 1L of deionized water.
- 8.1.1.5. Transfer water to the 1L vessel.
- 8.1.1.6. Add magnetic stir bar and stir to dissolve.
- 8.1.1.7. Adjust pH to 6 ±0.1.
- 8.1.1.8. Filter sterilize.
- 8.1.1.9. Label as: Buffer A, Equilibration Buffer, 20mM Phosphate, pH 6, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].

8.1.2. Buffer B: Elution Buffer, 20mM phosphate, pH 6, 1M NaCl

- 8.1.2.1. Weigh 29.2 ±0.2 grams NaCl.
- 8.1.2.2. Transfer to a 1L vessel.
- 8.1.2.3. Using a 500mL graduated cylinder, measure approximately 500mL of 20mM phosphate buffer pH 6.
- 8.1.2.4. Transfer to the 1L vessel with the NaCl.
- 8.1.2.5. Add magnetic stir bar and stir to dissolve.
- 8.1.2.6. Filter sterilize.
- 8.1.2.7. Label as: Buffer B, Elution Buffer, 20mM Phosphate, pH 6, 1M NaCl, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].

8.1.3. Cleaning Solution: 0.1M NaOH

- 8.1.3.1. Weigh 2.0 ±0.05 grams of NaOH.
- 8.1.3.2. Transfer NaOH to a 1L vessel.
- 8.1.3.3. Using a 500mL graduated cylinder, measure 500mL of deionized water.
- 8.1.3.4. Transfer to the 1L vessel.
- 8.1.3.5. Add magnetic stir bar and stir to dissolve.
- 8.1.3.6. Filter sterilize.
- 8.1.3.7. Label as: Cleaning Solution, 0.1M NaOH, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].

8.1.4. Buffer C: CHO Cell Culture Supernatant, pH 6

- 8.1.4.1. Adjust the pH of the CHO cell culture supernatant obtained during upstream processing of tPA to pH 6.
 - 8.1.4.1.1. Measure the pH of the CHO cell culture supernatant per pH meter SOP.

Title: Ion Exchange Chromatography of tPA SOP

- 8.1.4.1.2. If the initial pH is above 6 ± 0.1 , carefully add one drop of hydrochloric acid solution to the supernatant.
- 8.1.4.1.3. Observe the change in pH.
- 8.1.4.1.4. Repeat step 8.1.4.1. until the supernatant solution is pH 6 ± 0.1 .
- 8.1.4.2. Label as: Buffer C, CHO Cell Culture Supernatant, pH 6, Store: 2-8°C,
Dispose: drain, [date], [group], [initials].
- 8.2. Purge BioLogic LP Sytem with Buffer A and zero the UV monitor per the Biologic LP Chromatography System SOP**
- 8.3. Pour Column per the BioLogic LP Chromatography System SOP**
- 8.3.1. Use approximately 5mL of POROS HS resin.
- 8.4. Attach the column to the BioLogic LP per the BioLogic LP Chromatography System SOP**
- 8.5. Pack Column per the BioLogic LP Chromatography System SOP**
- 8.5.1. Place the line for Buffer A into the vessel containing Buffer A, Equilibration Buffer. Cover the opening of the vessel with laboratory film, such as Parafilm.
- 8.5.2. Use Method: IEX Pack:
- | | | | |
|---------|----------|----------|-----------|
| Step 1: | 0-5min | Buffer A | 1.0mL/min |
| Step 2: | 5-10min | Buffer A | 2.0mL/min |
| Step 3: | 10-20min | Buffer A | 3.0mL/min |
| Step 4: | 25-30min | Buffer A | 6.0mL/min |
- 8.6. Determine the HETP and h of the column per the BioLogic LP Chromatography System SOP.**
- 8.6.1. Use Method: IEX HETP
- | | | | |
|--------|---------|----------|---------|
| Step1: | 0-15min | Buffer A | 5mL/min |
|--------|---------|----------|---------|
- 8.6.2. The Dp of the bead is 0.05mm.
- 8.6.3. The expected HETP is approximately 0.1mm.
- 8.6.4. The h calculation should be less than 3. If h is greater than 3, the desired product may not bind the column efficiently. In this case it is best to re-pack the column.
- 8.7. Run Column per the BioLogic LP Chromatography System SOP.**
- 8.7.1. Place the lines for Buffers A, B and C into the vessels containing the appropriate buffers. Cover the opening of each vessel with laboratory film.
- 8.7.2. Use Method: IEX tPA
- | | | | |
|---------|-------------|----------|---------|
| Step 1: | 0 to 20min | Buffer C | 4mL/min |
| Step 2: | 20 to 40min | Buffer A | 4mL/min |
| Step 3: | 40 to 60min | Buffer B | 4mL/min |
| Step 4: | 60 to 80min | Buffer A | 4mL/min |
- 8.7.3. Collect 1-5mL of the flow through fraction when the first A.U. peak begins to plateau (approximately 5 minutes into the run).
- 8.7.4. Collect the entire elution fraction when the second A.U. peak BEGINS to appear (approximately 40 minutes into the run).
- 8.7.5. Store fractions at 2-8°C for SDS PAGE analysis.
- 8.8. Clean the Column per the BioLogic LP Chromatography System SOP.**
- 8.8.1. Place the lines for buffers A and B into the vessel containing Cleaning Solution, 0.1M NaOH. Cover the opening of the vessel with laboratory film.

Title: Ion Exchange Chromatography of tPA SOP

8.8.2. Use Method: IEX Clean

Step 1: 0 to 20min Buffer 50% B 4mL/min

8.9. Store the System per the BioLogic LP Chromatography System SOP.

9. Attachments: N/A

10. History:

Name	Date	Amendment
Sonia Wallman	2000	Initial Release
Deb Audino	7/2005	Changed from manual pump system to BioLogic LP system.
Deb Audino	051206	Removed steps associated with equipment operation to simplify the process SOP.
Deb Audino	31Aug07	Simplified the packing method.
Deb Audino	04Apr08	College name change
Kari Britt	31May09	Added labeling directions, directions for covering vessels with laboratory film, and directions for placing buffer lines into the appropriate buffer. Also made general grammar and formatting edits as needed throughout the document.