

Title: Tangential Flow and Diafiltration of HSA

Approvals:

Preparer: _____ Deb Audino _____ Date _____ 03Apr08 _____
Reviewer: _____ Bob O'Brien _____ Date _____ 03Apr08 _____

1. Purpose:

- 1.1. To concentrate and perform buffer exchange of protein product using tangential flow and diafiltration processes.

2. Scope:

- 2.1. Applies to the concentration and buffer exchange of HSA from *Pichia pastoris*.

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. pH meter SOP
- 4.2. Millipore Pellicon XL Tangential Apparatus SOP

5. Definitions:

- 5.1. Permeate- the material that passes through the membrane
- 5.2. Retentate- the material that does not pass through the membrane

6. Precautions: N/A

7. Materials:

- 7.1. NaH₂PO₄ (sodium phosphate monobasic, anhydrous)
- 7.2. Na₂HPO₄·7H₂O (sodium phosphate dibasic, heptahydrate)
- 7.3. 1L container
- 7.4. 1L graduated cylinder
- 7.5. 1L filter unit
- 7.6. pH Meter and pH paper
- 7.7. magnetic stir plate and stir bars
- 7.8. Millipore Pellicon XL Tangential Apparatus (BioMax 50 Regenerated Cellulose MWCO 10) and accessories.

8. Procedure:

- 8.1. Diafiltration Buffer Preparation (20mM Phosphate Buffer pH 7.1)
 - 8.1.1. Weigh out 0.80±0.02g NaH₂PO₄ and place into a 1L container.
 - 8.1.2. Weigh out 3.60±0.2g of Na₂HPO₄·7H₂O and place into the 1L container with the NaH₂PO₄.
 - 8.1.3. Using a 1L graduated cylinder, measure 1L of deionized water.
 - 8.1.4. Transfer water to the 1L flask.
 - 8.1.5. Add magnetic stir bar and stir to dissolve.
 - 8.1.6. Adjust pH to 7.1±0.1.
 - 8.1.7. Sterile Filter the solution and label container: 20mM Phosphate Buffer pH 7.1, [date], [initials], [group], storage: room temp, disposal: drain.
- 8.2. Set up the Millipore Pellicon XL Tangential Flow Filtration Apparatus per SOP.

Title: Tangential Flow and Diafiltration of HSA

- 8.3. Flush the Millipore Pellicon XL Tangential Flow Filtration Apparatus per SOP.
- 8.4. Precondition the Millipore Pellicon XL Tangential Flow Filtration Apparatus per SOP.
- 8.5. **Concentrate the sample.**
 - 8.5.1. Fill the feed container with sample to be concentrated.
 - 8.5.2. Place the ends of the retentate and feed tubes into the feed container and place the permeate tube into a separate container.
 - 8.5.3. Add stir bar to the sample and place on a stir plate. Slowly stir the sample.
 - 8.5.4. With thumbscrew still lightly tightened, turn on the pump, which has been set at a flow rate of 30-50 mL/min.
 - 8.5.5. Filter the solution until the desired volume is reduced 10-fold.
 - 8.5.6. Turn off the pump and empty the permeate container into a large bottle with cap. Label the bottle: HSA Permeate Waste, disposal: bleach then drain, [initials], [date].
- 8.6. **Perform a buffer exchange on the sample.**
 - 8.6.1. Add the 20mM Phosphate Buffer to the sample to bring the volume back to the pre-concentrated volume.
 - 8.6.2. Repeat step 8.5 until the pH of the concentrated retentate is 7.1±0.1 as measured with a pH meter.
- 8.7. **Retrieve the Sample**
 - 8.7.1. Turn the pump off and remove the feed tube from the feed container.
 - 8.7.2. Turn the pump on and pump all the retentate into the feed container.
 - 8.7.3. Turn the pump off.
 - 8.7.4. Remove the concentrated sample. Label as: Concentrated HSA, [date], [initials].
 - 8.7.5. Store in 2°C – 8°C refrigerator until column chromatography step.
- 8.8. Flush the Millipore Pellicon XL Tangential Flow Filtration Apparatus per SOP.
- 8.9. Clean the Millipore Pellicon XL Tangential Flow Filtration Apparatus per SOP.
- 8.10. Store the Millipore Pellicon XL Tangential Flow Filtration Apparatus per SOP.

9. Attachments: N/A

10. History:

Name	Date	Amendment
Sonia Wallman	2000	Initial Release
SCP	2003	
Deb Audino	04Nov05	Put into 2005 SOP format. Separated out tPA and HSA procedures. Simplified the preparation of the 20mM phosphate buffer step
Deb Audino	04Apr08	college name change