Batch Record: HSA Production from *Pichia pastoris*
Downstream Process
HSA Lot Number_____

Record Keeping Standards:

For each step in the batch record: the operator of the task will enter their initials (each operator has their own unique set of initials) and the date in the appropriate section(s) of the batch record. Another operator must initial and date in the appropriate section of the batch record to verify that the task was completed per SOP. No operator will verify their own work at any point. "If you didn't document it, you didn't do it!"

Batch records will be completed in blue or black ball point pen ONLY, and must be legible.

Any errors on a batch record will be crossed out with a single line through the error with the initials of the operator and the date. Corrections will be written in next to the crossed out error.

Use the following format to record dates: DDMMMYY. For July 10, 2006 use 10JUL06.

Use the 24 hour clock or "military time" to record time: 3:00pm would be written as 15:00.

Any and all deviations from a protocol or SOP, including abnormal results or retests performed, will be entered into the comments section at the end of each batch record. Be as detailed and specific as possible, include all steps taken before and/or after an abnormal reading, and provide an explanation for any deviations from a step.

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1. Solution and Buffer Preparation for Tangential Flow Filtration 20mM Phosphate Buffer pH 7.1 0.1M Sodium Hydroxide		
Calibrate pH meter per SOP with commercially prepared standard buffers (pH 7 and pH 4): pH Meter ID #_____ pH 7 Buffer Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ pH 4 Buffer Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____	Operator/Date	Verifier/Date
Weigh 0.80±0.02 grams sodium phosphate monobasic, anhydrous (NaH_2PO_4). Balance ID #:_____ Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Weigh 3.6±0.2 grams sodium phosphate dibasic, heptahydrate (Na_2HPO_4-$7H_2O$). Balance ID #:_____ Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Dissolve sodium phosphate monobasic anhydrous with the sodium phosphate dibasic heptahydrate in approximately 1L of deionized water using magnetic stir bar. Volume of water added: _____ mL	Operator/Date	Verifier/Date
Adjust 20mM Phosphate Buffer to pH 7.1±0.1. pH_____	Operator/Date	Verifier/Date
Sterile Filter solution and label container: 20mM Phosphate Buffer pH 7.1, [date], [initials], [group], storage: room temp, disposal: drain.	Operator/Date	Verifier/Date
Weigh 4.0±0.2 grams of sodium hydroxide (NaOH): Balance ID #:_____ Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris*
Downstream Process
HSA Lot Number_____

Dissolve NaOH in approximately 1L of deionized water using magnetic stir bar. Volume of water added: _____ mL	Operator/Date	Verifier/Date
Sterile filter solution and label container: 0.1M NaOH, [date], [initials], [group number], storage: room temp, disposal: adjust to pH 7 then drain.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date
2. Set up, flush, and precondition the tangential flow filtration apparatus.		
Obtain Millipore Pellicon XL Tangential Filter from 2-8°C. Millipore Pellicon XL ID#_____ **Obtain** Millipore peristaltic pump. Pump ID# _____	Operator/Date	Verifier/Date
Flush system per Millipore Pellicon XL Tangential Flow Filter SOP. While flushing, set the flow rate to 30-50ml/min. Note: DO NOT adjust speed dial once the correct flow rate is achieved. Flow Rate: _____ Pump Speed: _____	Operator/Date	Verifier/Date
Check the pH of the system after flushing. pH of the retentate_____	Operator/Date	Verifier/Date
Precondition the system per Millipore Pellicon XL Tangential Flow Filter SOP with 20mM phosphate buffer. Volume of buffer collected:_____mL	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris*
Downstream Process
HSA Lot Number_____

3. Concentrate and buffer exchange the sample.		
Pour Pichia supernatant into the feed container. Concentrate per Tangential Flow and Diafiltration of HSA SOP. Initial supernatant volume: _____mL Final supernatant volume: _____mL	Operator/Date	Verifier/Date
Buffer exchange the sample per the Tangential Flow and Diafiltration of HSA SOP. After each concentration step is complete, check pH of the retentate. Once the pH of the concentrated retentate is 7.1, TFF is complete Final pH of the concentrated sample (with pH meter):_____	Operator/Date	Verifier/Date
Label container: Filtered Pichia Supernatant, [date], [initials], [group number], storage: 2-8°C, dispose: autoclave and drain. Store for chromatography purification.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date
4. Flush, clean and store the tangential flow filtration apparatus.		
Flush the apparatus with biopure water per the Millipore Pellicon XL Tangential Flow Filter SOP.	Operator/Date	Verifier/Date
Clean the apparatus with 0.1M NaOH per SOP until the pH of the retentate is greater than 10. pH of the retentate:_____	Operator/Date	Verifier/Date
If storing unit, leave lines filled with 0.1M NaOH and label unit with status tag: Stored: 0.1M NaOH, [date], [initials].	Operator/Date	Verifier/Date
If not storing unit, flush lines with biopure water until the pH of the retentate is <7.2. Label unit: Cleaned/Rinsed: 0.1M NaOH/biopure water, [date], [initials]. pH of the retentate:_____	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris*
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HSA Lot Number_____

5. Solution and Buffer Preparation for Affinity Chromatography of HSA Buffer A: Equilibration Buffer, 20mM Phosphate, pH 7.1. Buffer B: Elution Buffer, 20mM Phosphate pH 7.1, 1M NaCl Cleaning Solution: 2.5mM NaOH		
Calibrate pH meter per SOP with commercially prepared standard buffers (pH 7 and pH 4): pH Meter ID #_____ pH 7 Buffer Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ pH 4 Buffer Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____	Operator/Date	Verifier/Date
Weigh 0.80±0.02 grams sodium phosphate monobasic, anhydrous (NaH_2PO_4). Balance ID #:_____ Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Weigh 3.6±0.2 grams sodium phosphate dibasic, heptahydrate (Na_2HPO_4-$7H_2O$). Balance ID #:_____ Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Dissolve sodium phosphate monobasic anhydrous with the sodium phosphate dibasic heptahydrate in approximately 1L of deionized water using magnetic stir bar. Volume of water added: _____ mL	Operator/Date	Verifier/Date
Adjust 20mM Phosphate Buffer to pH 7.1±0.1. pH_____	Operator/Date	Verifier/Date
Sterile Filter solution and label as: Buffer A, Equilibration Buffer, 20mM Phosphate, pH 7.1, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris*
Downstream Process
HSA Lot Number_____

Weigh 29.2 ±0.2 grams NaCl. Balance ID #:_____ Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Dissolve in approximately 500mL of Equilibration Buffer A using magnetic stir bar. Volume of Buffer A added _____ mL	Operator/Date	Verifier/Date
Sterile filter solution and label as: Buffer B, Elution Buffer, 20mM Phosphate, pH 7.1, 1M NaCl, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].	Operator/Date	Verifier/Date
Weigh 0.10 ±0.02 grams of NaOH. Balance ID #:_____ Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Dissolve in approximately 500mL deionized water using magnetic stir bar. Volume of water added _____ mL	Operator/Date	Verifier/Date
Sterile filter solution and label as: Cleaning Solution, 2.5mM NaOH, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].	Operator/Date	Verifier/Date
Label the concentrated HSA in 20mM Phosphate buffer, pH 7.1 as: Buffer C, Concentrated HSA in 20mM Phosphate buffer, pH 7.1, Store: 2-8°, Dispose: Drain, [date], [group], [initials].	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

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6. Purge BioLogic LP System, Pour Column and Attach to Biologic LP System		
Calibrate pump if necessary per the BioLogic LP Chromatography System SOP. **Verify** that **1.6mm** tubing is in the pump. Change tubing if necessary. Tubing changed: Yes / No (Circle) If the tubing was changed, adjust the platen and calibrate the pump per BioLogic LP SOP. Platen adjusted: Yes / No (Circle) Pump recalibrated: Yes / No (Circle)	Operator/Date	Verifier/Date
Purge the BioLogic LP system with Buffer A per the Biologic LP Chromatography System SOP.	Operator/Date	Verifier/Date
Place each buffer line into a container filled with Buffer A (Equilibration Buffer).	Operator/Date	Verifier/Date
Zero the UV monitor per the Biologic LP Chromatography System SOP.	Operator/Date	Verifier/Date
Add approximately 5mL of Affi-Gel Blue beads to column per BioLogic LP Chromatography System SOP. Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Volume of Affi-Gel Blue added _____ mL	Operator/Date	Verifier/Date
Attach the column to the BioLogic LP per the BioLogic LP Chromatography System SOP. BioLogic LP ID#_____ Amicon Vantage-L-Column ID#_____	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris*
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HSA Lot Number_____

7. Pack the Column and Determine HETP and h		
Pack column per the BioLogic LP Chromatography System SOP using Method: Affi Pack.	Operator/Date	Verifier/Date
Place the line for Buffer A into the vessel containing Buffer A, Equilibration Buffer. Cover the vessel opening with a laboratory film, such as Parafilm.	Operator/Date	Verifier/Date
Determine column volume per the BioLogic LP Chromatography System SOP. CV = π(bed height in cm)(radius of column in cm)^2 Write out CV calculation in this space: Bed Height: _____ Column Volume: _____	Operator/Date	Verifier/Date
Produce chromatogram needed to determine HETP and h per BioLogic LP Chromatography System SOP using Method: Affi HETP. Volume of Elution Buffer B loaded:_____mL	Operator/Date	Verifier/Date
Determine HETP of the column per BioLogic LP Chromatography System SOP and **attach** chromatogram to batch record. Dp = 0.3mm for Affi-Gel Blue beads. Write out HETP and h calculations in this space: HETP value:_____mm h value:_____	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: HSA Production from *Pichia pastoris*
Downstream Process
HSA Lot Number_____

8. Run Column		
Run column per the BioLogic LP Chromatography System SOP using Method: Affi HSA.	Operator/Date	Verifier/Date
Place the lines for Buffers A, B, and C into the vessels containing the appropriate buffer. Cover the vessels with laboratory film.	Operator/Date	Verifier/Date
Store fractions at 2 – 8ºC for SDS PAGE Analysis.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date
9. Clean and Store BioLogic LP Chromatography System		
Clean the column per the BioLogic LP Chromatography System SOP using Method: Affi Clean. Use Cleaning Solution, 0.1M NaOH for Buffers A and B.	Operator/Date	Verifier/Date
Clean and store the BioLogic LP Chromatography System per the BioLogic LP Chromatography System SOP. Column Storage (Check one): Left on Biologic System _____ Disconnected and stored at room temp. _____ Disconnected and stored at 2-8°C _____ Disassembled _____	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date