

Batch Record: tPA Production from CHO Cells Downstream Process
tPA 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: DDMMYY. 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 Buffer A: Equilibration Buffer, 20mM phosphate, pH 6. Buffer B: Elution Buffer, 20mM phosphate pH 6, 1M NaCl Cleaning Solution: 0.1M NaOH Adjust cell culture supernatant to pH 6.		
Calibrate pH meter per SOP with commercially prepared standard buffers (pH 7 and pH 4): pH Meter ID # _____ <u>pH 7 Buffer</u> Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____ <u>pH 4 Buffer</u> Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____	Operator/Date	Verifier/Date
Weigh 2.10 ±0.05 grams of sodium phosphate monobasic anhydrous. Balance ID #: _____ Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____ Amount weighed: _____ grams	Operator/Date	Verifier/Date
Weigh 0.66 ±0.02 grams of sodium phosphate dibasic heptahydrate. 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 Equilibration Buffer A to pH 6.0 ±0.1. pH _____	Operator/Date	Verifier/Date
Sterile filter solution and label as: Buffer A, Equilibration Buffer, 20mM Phosphate, pH 6, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].	Operator/Date	Verifier/Date

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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 6, 1M NaCl, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].	Operator/Date	Verifier/Date
Weigh 2.0 ±0.05 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, 0.1M NaOH, Store: Room Temperature, Dispose: Drain, [date], [group], [initials].	Operator/Date	Verifier/Date
Adjust pH of CHO cell culture supernatant to pH 6 ±0.1. pH _____	Operator/Date	Verifier/Date
Label CHO cell culture supernatant as: Buffer C, CHO Cell Culture Supernatant, pH 6, Store: 2-8°C, Dispose: drain, [date], [group], [initials].	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

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2. Purge BioLogic LP System, Pour Column and Attach to Biologic LP System		
<p>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 one.)</p> <p>If the tubing was changed, adjust the platen and calibrate the pump per BioLogic LP SOP. Platen adjusted: Yes / No (Circle one.) Pump recalibrated: Yes / No (Circle one.)</p>	Operator/Date	Verifier/Date
<p>Purge the BioLogic LP system with Buffer A per the Biologic LP Chromatography System SOP.</p>	Operator/Date	Verifier/Date
<p>Place each buffer line into a container filled with Buffer A (Equilibration Buffer).</p>	Operator/Date	Verifier/Date
<p>Zero the UV monitor per the Biologic LP Chromatography System SOP.</p>	Operator/Date	Verifier/Date
<p>Add approximately 5ml of POROS 50 HS resin to column per BioLogic LP Chromatography System SOP. Volume of POROS 50 HS added: _____ mL Amicon Vantage-L-Column ID#: _____</p>	Operator/Date	Verifier/Date
<p>Attach the column to the BioLogic LP per the BioLogic LP Chromatography System SOP. BioLogic LP ID#: _____</p>	Operator/Date	Verifier/Date
<p>Comments:</p>	Operator/Date	Verifier/Date

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3. Pack the Column and Determine HETP and h		
Pack column per the BioLogic LP Chromatography System SOP using Method: IEX Pack.	Operator/Date	Verifier/Date
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.	Operator/Date	Verifier/Date
Determine column volume per the BioLogic LP Chromatography System SOP. $CV = \pi(\text{bed height in cm})(\text{radius of column in cm})^2$ 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: IEX HETP. Volume of 0.8M NaCl loaded _____ mL	Operator/Date	Verifier/Date
Determine HETP of the column per BioLogic LP Chromatography System SOP and attach chromatogram to batch record. Dp = 0.05mm for POROS HS resin. Write out HETP and h calculations in this space: HETP value: _____ mm h value: _____	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

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4. Run Column		
Run column per the BioLogic LP Chromatography System SOP using Method: IEX tPA.	Operator/Date	Verifier/Date
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.	Operator/Date	Verifier/Date
Store fractions at 2 – 8°C for SDS PAGE Analysis.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date
5. Clean and Store BioLogic LP Chromatography System		
Clean the column per the BioLogic LP Chromatography System SOP using Method: IEX Clean.	Operator/Date	Verifier/Date
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.	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 2-8C _____ Disassembled _____	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date