Batch Record: tPA Production from CHO Cells Upstream 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: 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.

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

1. Media Preparation		
Clean, assemble and autoclave two 100mL Bellco Spinner flasks per SOP. Spinner flask ID#_____Spinner flaskID#_____	Operator/Date	Verifier/Date
Obtain sterile Fetal Bovine Serum (FBS). Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____	Operator/Date	Verifier/Date
Obtain sterile Ham's F12 Medium Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____	Operator/Date	Verifier/Date
Sterilely add 90mL ± 1 mL of Ham's F12 Medium to a spinner flask. Repeat with the second spinner flask 100mL spinner flask ID#_____Vol of Ham's F12_____mL 100mL spinner flask ID#_____Vol of Ham's F12_____mL	Operator/Date	Verifier/Date
Sterilely add 10mL ± 1 mL of FBS to each spinner flask. 100mL spinner flask ID#_____Vol of FBS_____mL 100mL spinner flask ID#_____Vol of FBS_____mL	Operator/Date	Verifier/Date
Label spinner flasks as 90% Ham's F12, 10% FBS, [date], [group#], [operator initials].	Operator/Date	Verifier/Date
Place spinner flasks containing CHO cell media in the CO_2 incubator. Set the speed of the magnetic stirrer to the maximum setting that ensures an even vortexing of the culture without foaming.	Operator/Date	Verifier/Date
Verify that CO_2 is set to 5±0.5% and that temperature is set to 37±0.5ºC. CO_2_____% Temperature _____ ºC	Operator/Date	Verifier/Date
Check media for contamination after a minimum of 24 hrs. Elapsed Incubation Time _____ 100mL spinner flask ID_____ Contamination: Y / N (Circle) 100mL spinner flask ID_____ Contamination: Y / N (Circle)	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

2. Inoculation of Spinner Flasks		
Pre-warm the spinner Flasks containing CHO Cell Culture Medium at 37° C ± 0.5°C overnight.	Operator/Date	Verifier/Date
Remove two vials of CHO cells from storage in the -86°C freezer. Vial ID:_____ _____ Vial ID:_____ _____	Operator/Date	Verifier/Date
Sterilely transfer the entire contents of each 1mL vial of thawed CHO Cells into each of the previously prepared Spinner Flask containing 100mL CHO Cell Culture Medium using a 2mL sterile pipette. **Swirl** to mix.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number____________________

100mL Spinner Flask ID#___________________________________

TIME (hours)	OD 650nm	pH	LIVE CELL Count	DEAD CELL Count	Viable cells/mL	Percent Viability	GLUCOSE (mg/dL)	LACTATE (mmol/L)
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier

Batch Record: tPA Production from CHO Cells Upstream Process

tPA Lot Number____________________

100mL Spinner Flask ID#________________________________

TIME (hours)	OD 650nm	pH	LIVE CELL Count	DEAD CELL Count	Viable cells/mL	Percent Viability	GLUCOSE (mg/dL)	LACTATE (mmol/L)
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

3. Solution and Buffer Preparation 500mL 1M ($NaHCO_3$) sodium bicarbonate 100mL of 1X PBS Phosphate buffered Saline		
Weigh out 21.0 ± 1 grams of ($NaHCO_3$) sodium bicarbonate. Label container: 1M $NaHCO_3$, [date], [initials], [group number], storage: room temp, disposal: drain. Balance ID #:_____ Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount weighed:_____grams	Operator/Date	Verifier/Date
Dissolve $NaHCO_3$ in 250 ± 5mL of deionized water using magnetic stirrer. Volume of water added _____ mL	Operator/Date	Verifier/Date
Dilute 10 ± 0.5mL of 10X stock solution, with 90 ± 5mL of deionized water in 100mL bottle using magnetic stirrer. Label container: 1X PBS, [date], [initials], [group number], storage: room temp, disposal: drain. Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Volume of 10x PBS added:_____mL Volume of water added: _____mL	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

4. Assemble/Autoclave Bioreactor		
4.1. Assemble Vessel Stand		
Inspect the integrity of the large O-rings on the vessel stand and headplate. Replace if worn or cracked. Bioreactor ID #_____ Vessel stand O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.) Headplate O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
4.2. Assemble Headplate-Underside		
Inspect the integrity of the O-rings on the harvest tube, sparger, and the thermowell. Harvest tube O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.) Sparger O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.) Thermowell O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Attach harvest tube, sparger and thermowell. **Verify** that the sparger tube is aligned beneath the stirrer impeller.	Operator/Date	Verifier/Date
4.3. Attach Headplate to Vessel Stand.		
Place the headplate onto the vessel stand, positioning the holes on the outer edge of the headplate with the bolts on the vessel stand.	Operator/Date	Verifier/Date
Place the sample bottle assembly onto the bolt located by the 3 addition port and attach with a mill fastener.	Operator/Date	Verifier/Date
Secure the headplate with the 5 mill fasteners.	Operator/Date	Verifier/Date
4.4. Assemble Headplate – Topside		
Inspect the integrity of the O-ring in the condenser port of the headplate. Replace if worn or cracked. Condenser port O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced?: Yes / No (Circle one.)	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

Inspect the black seal at the bottom of the condenser underneath the retainer nut. Replace if worn or cracked. Condenser black seal worn or cracked? Yes / No (Circle one.) Black seal replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Attach condenser to headplate	Operator/Date	Verifier/Date
Remove protective cap from the bottom of the DO probe and inspect screen. Replace if damaged. Protective screen damaged? Yes / No (Circle one.) Protective screen replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Unscrew the membrane module from the bottom housing of the probe tip. Inspect the integrity of the O-ring. Replace if worn or cracked. O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Replenish DO electrolyte with O_2 electrolyte solution.	Operator/Date	Verifier/Date
Inspect the integrity of the O-ring at the top of the stainless steel DO probe. Replace if worn or cracked. O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Inspect the black seal at the top of the DO probe under the retainer nut. Replace if worn or cracked. Black seal worn or cracked? Yes / No (Circle one.) Black seal replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Attach DO probe to the headplate.	Operator/Date	Verifier/Date
Calibrate the pH probe. pH 7 Buffer Manufacturer:_____ Catalog number: _____ Lot number: _____ Expiration date:_____ pH 4 Buffer Manufacturer:_____ Catalog number: _____ Lot number: _____ Expiration date:_____	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

Record pH calibration values. pH 7.00 standard: pH value _____ temp_____ pH 4.00 standard: pH value _____ temp_____ Slope from the Display _____Expected value: 0.95-1.05 Offset from the Display_____Expected value: < ±0.3	Operator/Date	Verifier/Date
Inspect the integrity of the O-ring at the top of the pH probe. Replace if worn or cracked. O-ring worn or cracked? Yes / No (Circle one.) O-ring replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Inspect the black seal at the top of the pH probe under the retainer nut. Replace if worn or cracked. Black seal worn or cracked? Yes / No (Circle one.) Black seal replaced? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Attach pH probe to the headplate.	Operator/Date	Verifier/Date
4.5. Attach Filters and Tubing		
Place silicone tubing on the Sparger tube,Condenser top outlet, and CO_2 overlay port. **Use** a small piece of silicon tubing to connect together 2 of the ports on the 3 port addition. **Connect** the pharmed tubing from the feed bottle to the 3 addition port. **Connect** the sample bottle tubing to the harvest tube.	Operator/Date	Verifier/Date
Clamp off all tubing (near the headplate) except the condenser top outlet. The condenser top outlet must remain unclamped to release pressure during autoclaving.	Operator/Date	Verifier/Date
Close all open ends with glass wool and cover with aluminum foil (including the harvest tube and sample bottle assembly tubing).	Operator/Date	Verifier/Date
Autoclave per SOP. Autoclave at 121°C for 20 minutes, using slow exhaust.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

5. Media Preparation and Addition / Run Preparation		
Place the bioreactor and a sterile funnel in the Biological Safety Cabinet and expose to UV light for 20-30 minutes.	Operator/Date	Verifier/Date
Add approximately 6mL of 200mM Glutamine and 10mL of 10mg/mL gentamycin to a 1L bottle of ProCHO4 media. Pour into bioreactor. ProCHO4 media: Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Glutamine: Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount added:_____mL Gentamicin: Manufacturer:_____Catalog number: _____ Lot number: _____Expiration date:_____ Amount added:_____mL	Operator/Date	Verifier/Date
Verify that glycerol has been added to the thermowell with the Pt-100 temperature probe. Add more if necessary.	Operator/Date	Verifier/Date
Verify that thermal blanket is wrapped around the vessel and plugged into the ADI 1025 unit.	Operator/Date	Verifier/Date
Input the following limits per the process SOP and activate the control loops. **Parameter** **Upper limit** **Lower limit** pH 7.3 7.1 Temperature 38 36 DO 52 48 Temperature 38 36 Agitation 76 74	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

Calibrate DO probe per Applikon Bioreactor Operation SOP. **Note:** Allow DO probe to polarize for at least 6 hours before performing calibration. **Record** slope: _____ Expected values are: 8-15 at 37°C or 10-20 at 25°C	Operator/Date	Verifier/Date
Turn on Air supply at regulator Tank pressure _____ Tank Volume_____	Operator/Date	Verifier/Date
Turn on CO_2 supply at regulator to the bioreactor. Tank pressure _____ Tank Volume_____	Operator/Date	Verifier/Date
Check the media for contamination before inoculation. Contamination? Yes / No (Circle one.)	Operator/Date	Verifier/Date
Inoculate bioreactor when the 100mL suspension culture of CHO cells reaches a concentration of about 1,000,000 cells/mL. Volume of culture added:_____	Operator/Date	Verifier/Date
Turn on computer and open BioXpert Lite software per Applikon Bioreactor Operation SOP. **Name** the file. File Name: _____	Operator/Date	Verifier/Date
Ensure the computer is communicating with the controller per the Applikon Bioreactor Operation SOP. **Click** the OK button to begin the data collection process. **IMPORTANT** – In the On-Line Session window **DO NOT CLICK ON *END***. This will end the on-line session and stop collecting data.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number____________________

Applikon Bioreactor ID#____

TIME (hours)	OD 650nm	pH	LIVE CELL Count	DEAD CELL Count	Viable cells/mL	Percent Viability	GLUCOSE (mg/dL)	LACTATE (mmol/L)
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier
Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier	Operator/verifier

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

6. Ending a Run		
Save the file for the run. File Name: _____	Operator/Date	Verifier/Date
Turn off each control loop. Turn off the supply of Air the ADI1025 controller. Turn off the supply of CO_2 supplied to the ADI1025 controller.	Operator/Date	Verifier/Date
Aseptically remove the culture through the harvest port.	Operator/Date	Verifier/Date
Clean the pH , DO, and the Pt-100 probes with a 10% bleach solution, and rinse with DI water. Place protective caps on the pH probe. Place protective caps on the DO probes.	Operator/Date	Verifier/Date
Clean the bioreactor.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date
7. Harvest and Preparation of Working Cell Bank		
Using a 25mL sterile pipet, divide the 500mL suspension culture into about 20 sterile 30mL centrifuge tubes. (about 25mL per tube).	Operator/Date	Verifier/Date
Centrifuge tubes for 10min at 2000rpm. (If using the Sigma 2K15 choose program 75). BE SURE TO BALANCE TUBES WHEN LOADING ROTOR.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

8. Prepare storage menstrum:		
In a container capable of holding >50mL add 40mL ± 1mL of Ham's F12 manufacturer: _____ lot number: _____ expiration date: _____ volume Ham's F12:_____	Operator/Date	Verifier/Date
Into the same container add 5mL ± 0.5mL of FBS manufacturer: _____ lot number: _____ expiration date: _____ volume FBS:_____	Operator/Date	Verifier/Date
Into the same container add 5mL ± 0.5mL of glycerol manufacturer: _____ lot number: _____ expiration date: _____ volume FBS:_____	Operator/Date	Verifier/Date
Filter sterilize and label bottle as CHO storage Menstrum with the date.	Operator/Date	Verifier/Date
Following centrifugation, decant tPA containing medium into sterile 250mL bottles. **Label** bottles as unpurified tPA in Ham's F12/FBS and date. **Store** supernatant in the refrigerator at 2-8°C.	Operator/Date	Verifier/Date
Add about 1mL of storage menstrum to each centrifuge tube to resuspend the pelleted CHO cells. **Sterilely dispense** 1mL ± 0.1mL aliquots into sterile 1.5mL cryovials. **Label** in the following manner using a cryopen: CHO (ATCC CRL-9606), [DATE], [INITIALS]. **Place** in a styrofoam tube rack, label container same as cryovials. **Store** at -85°C.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

Batch Record: tPA Production from CHO Cells Upstream Process
tPA Lot Number_____

9. Prepare Growth Curves		
Plot OD, pH, viable cells, glucose, and lactatevs. time (use 2 y-axes). Attach graph to Batch Record.	Operator/Date	Verifier/Date
Determine growth rate and doubling time of the 50mL and 500mL cultures (Show calculation) Growth Rate 100mL ID#_____is_____ Growth Rate 100mL ID#_____is _____ Growth Rate bioreactor ID#_____is _____	Operator/Date	Verifier/Date
Send samples to QC Chemistry department for ELISA and Activity Assays.	Operator/Date	Verifier/Date
Attach QC data to the batch record.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date