Montgomery County Community College
340 DeKalb Pike
Blue Bell, PA 19422



**Batch Record: Batch Culture of NISTCHO Cells for Production of cNISTmAb**
**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.

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, 2017 use 10JUL17.

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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| **1.0. Media hold, Inoculation and Shake flask Culture** | | |
|---|---|---|
| **1.1.** Obtain sterile 250ml Erlenmeyer PETG flat base shake flask with vent cap.<br>Manufacturer: __________________________ Catalog number: _______________<br>Lot number: ___________________________ Expiration date: __________________ | Operator/Date | Verifier/Date |
| **1.2. Obtain sterile T25 tissue culture flask**.<br>Manufacturer: __________________________ Catalog number: _______________<br>Lot number: ___________________________ Expiration date: __________________ | Operator/Date | Verifier/Date |
| **1.3. Obtain sterile EX-CELL® CD CHO Fusion Media**<br>Manufacturer: __________________________ Catalog number: _______________<br>Lot number: ___________________________ Expiration date: __________________ | Operator/Date | Verifier/Date |
| **1.4. Preparation of shake flask and Blank for media hold and media hold**<br>1.4.1. Prepare the Biological safety cabinet (BSC) per SOP with the required material to prepare shake flank and blank for media hold<br>1.4.2. Place the sterile 250ml Erlenmeyer PETG flat base shake flask with vent cap and sterile T25 vented tissue culture flask in the prepared BSC after swabbing with 70% Ethanol.<br>1.4.3. Place the sterile EX-CELL® Advanced CHO Fusion Medium (expansion medium) in the BSC<br>1.4.4. Aseptically transfer 98ml of CHO Fusion medium to the shake flask<br>1.4.5. Aseptically transfer 20ml of CHO Fusion medium to the T25 flask<br>1.4.6. Label the shake flask as NISTCHO, [date], [team name].<br>1.4.7. Label the T25 tissue culture flask as BLANK, [date], [team name]<br>1.4.8. Place the prepared shake flask in the $CO_2$incubator. Set the shaking speed at 125rpm.<br>1.4.9. Place the prepared T25 tissue culture flask in the top shelf of the $CO_2$incubator<br>1.4.10. Verify that the temperature is 37 ±0.5°C and the percentage $CO_2$is 5±0.5% and the shaking speed is 125rpm<br>1.4.11. Record the time and date below:<br>Incubation start time: ________ Incubation start Date:________<br>1.4.12. Perform the media hold for 24 hrs. ±2 hrs.<br>1.4.13. Check the media for contamination after 24 hrs.<br>Elapsed time: ____________<br>Contamination: Yes / No (circle one) | Operator/Date | Verifier/Date |
| **1.5. Inoculation of shake flask**<br>1.5.1. Prepare a biological safety cabinet per SOP with required materials to perform inoculation.<br>1.5.2. Verify the Thawstar is turned on and the ThawSTAR® CFT2 transporter has a prechilled transporter core with handle. | Operator/Date | Verifier/Date |

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| 1.5.3. Remove two vials of NISTCHO cells from the storage in the -150°C freezer and place them in the prepared Thawstar transporter. Record removal of the two vials in the -150°C freezer log. **Each vial should contain 1.3 x $10^7$ cells in 1 ml to obtain an initial culture concentration of 2.6 x $10^5$cells/ml after inoculation.**<br>Vial ID __________ Vial ID __________<br>Cell Concentration __________ Cell Concentration __________<br>Cryopreservation date __________ Cryopreservation date __________<br>1.5.4. Thaw one vial at a time rapidly using the Thawstar and leaving the non-thawing vial in the thawstar transporter.<br>1.5.5. Spray the thawed vial with 70% isopropanol/EtOH and place it in the biological safety cabinet. Meanwhile place the second vial of the cells in the thawstar.<br>1.5.6. Quickly remove the shake flask from the incubator spray and place it in the BSC for inoculation.<br>1.5.7. Aseptically transfer the entire content of the 1 ml vial of the thawed NISTCHO cells into the shake flask with a 2ml sterile serological pipette.<br>1.5.8. Spray the thawed second vial with 70% isopropanol/EtOH and place them in the biological safety cabinet.<br>1.5.9. Aseptically transfer the entire content of the second 1 ml vial of the thawed NISTCHO cells into the shake flask with a 2ml sterile serological pipette. Do not add cells to the T25 tissue culture flask labelled BLANK.<br>1.5.10. Swirl to mix. Place the shake flask back in the incubator set at 37°C with 5% $CO_2$ and 125 rpm for 15 minutes.<br>1.5.11. Take a day 0 sample following the procedure described in 1.6. | | |
| **Comments** | Operator/Date | Verifier/Date |
| **1.6. Monitoring/Sampling the cell culture**<br>In BSC, 1.2ml sample of culture will be removed 15 minutes after inoculation of the shake flask this will be Day 0 sample. This step will be repeated at specified time point to monitor cell growth, viability, and culture condition. Analyze samples from each time point using tests for:(1) optical density at 650 nm, (2) viable cell density and viability, (3) pH, (4) glucose/lactate concentration. Samples will be tested every 24hrs. ±2 hrs. until day 7 (cell density should be 5.5-6.5 x $10^6$ cells/ml), the culture can than be used to inoculate the bioreactor.<br>1.6.1. **Sampling the culture**<br>1.6.1.1. Prepare a biological safety cabinet per SOP | Operator/Date | Verifier/Date |

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| 1.6.1.2. Collect the following items, spray with 70% EtOH and place in Biological Safety Cabinet<br>• 1.5ml Microfuge tube labelled NISTCHO<br>• 1.5ml microfuge tube labelled blank<br>• microfuge tube holder<br>• 1-Pipette aid<br>• 2, 2ml individually wrapped serological pipette<br>1.6.1.3. Remove BLANK T25 tissue culture flask from CO2 incubator, spray 70% IPA and place in biological safety cabinet<br>1.6.1.4. Using aseptic technique, remove 1.1 mL from the BLANK T25 flask and place into a 1.5 mL microfuge tube labeled blank<br>1.6.1.5. Remove shake flask labelled NISTCHO with your team's name, spray and place in BSC<br>1.6.1.6. Using aseptic technique, remove 1.2 mL of culture from the NISTCHO flask and transfer to the 1.5ml microfuge labelled NISTCHO. Be sure to mix the culture and remove sample form the middle of the culture suspension<br>1.6.1.7. Return NISTCHO flask and BLANK T25 tissue culture flask to the CO2 incubator<br>1.6.1.8. On the bench top mix the 1.2 mL cell suspension by inverting the 1.5 mL tube several times. Transfer 100 µl of cell suspension to the tube labelled "cell count" | | |
| 1.6.2. **Cell concentration and viability determination**<br>1.6.2.1. Using the 100 µl of cell suspension from microfuge tube labelled "cell count" from the step above determine cell count and cell viability using "Operation of Logos Biosystems Luna-FL Fluorescence Cell Counter for Fluorescence Cell Counting SOP"<br>1.6.2.2. Record the viable cell count cells/ml and % viability in the shake flask data table | Operator/Date | Verifier/Date |
| 1.6.3. **pH Measurement**<br>1.6.3.1. Calibrate pH meter with the microprobe per SOP<br>1.6.3.2. Place the microprobe into the 1.5ml tube containing 1.1ml of culture<br>1.6.3.3. Measure and record pH<br>1.6.3.4. Rinse probe with Milli Q water, 70% EtOH, followed by MilliQ water<br>1.6.3.5. Store the pH microprobe in the pH storage solution | Operator/Date | Verifier/Date |
| 1.6.4. **OD Measurement at 650 nm**<br>Collect<br>• Two cuvettes and a cuvette holder<br>• P1000 micropipette and tips<br>• Microfuge tube labelled G/L and initials<br>1.6.4.1. Turn on the Genesys 180 spectrophotometer 5 minutes before measuring the absorbance. | Operator/Date | Verifier/Date |

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| 1.6.4.2. The main menu will be displayed once the power on is completed (about 2 to 3 minutes)<br>1.6.4.3. Select the "Fixed" icon on the main screen.<br>1.6.4.4. Select the "+" icon located in the bottom right corner to create a new protocol.<br>1.6.4.5. Change the method name to "vessel name, team name and date" and press done.<br>1.6.4.6. Press "SETUP" located on the top right corner of the screen.<br>1.6.4.7. Select "8 cell changer" and enter "1" for the number of cell changer.<br>1.6.4.8. 1.10.8. Unselect 8-3 and select the "←" in the top left to return to the SETUP screen.<br>1.6.4.9. Change the wavelength to 650nm by pressing the number displayed and enter 650.<br>1.6.4.10. Open the sample compartment lid. Align the white arrow on the 8-cell changer with the arrow on the sample compartment by manually turning the changer with the blue knob.<br>1.6.4.11. Label one cuvette "B" and one cuvette "S" (Note: Don't touch the cuvette below the frosted area)<br>1.6.4.12. Transfer 1 ml of blank to the cuvette labelled "B" and 1 ml of sample to the cuvette labelled "S" using p1000 micropipette (Note: mix the sample by pipetting up and down gently before transferring to the cuvette to take a representative sample)<br>1.6.4.13. Place the cuvette labelled blank in sample holder 1 and cuvette labelled sample in sample holder 2. Load the cuvettes such that the longer path length (10mm) is perpendicular to the white arrow of the sample compartment. Close the sample compartment lid.<br>1.6.4.14. Press "continue" on the display screen.<br>1.6.4.15. Record the measured absorbance in the data table for the shake flask<br>1.6.4.16. Select three dots icon on the top right of the screen. Select print icon on the screen to print the results.<br>1.6.4.17. When done exit the menu by pressing "X" on the to left side of the screen. Press "end experiment".<br>1.6.4.18. Remove cuvettes from the spectrophotometer.<br>1.6.4.19. Transfer the sample from the cuvette to a 1.5 ml sterile microfuge tube labelled "G/L" and team name. Centrifuge the tube for 5 minutes in the microcentrifuge.<br>1.6.4.20. Transfer 980µl of the supernatant to a new sterile 1.5 ml microfuge tube. Label the tube with day of the culture, date, type of vessel (shaker or bioreactor) and team initials<br>1.6.4.21. Add bleach to the tube with cell pellet and sample cuvette and discard in the biohazard waste. Drain the blank solution in the sink and discard the blank cuvette in the biohazard waste. | | |
| **Comments:** | | |

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| 1.6.5. **Glucose and Lactate Measurement**<br>Collect:<br>• Microfuge tube labelled G/L from step 1.6.4.20<br>1.6.5.1. Measure Glucose and Lactate concentration using the YSI Bioanalyzer by following "SOP Measurement of Glucose and Lactate concentration in Media using YSI 2500 Bioanalyzer<br>1.6.5.2. Following glucose/lactate determination record the data in the shake flask table. Store the sample tube at 2-8°C in a microfuge storage box labeled with team name | | |
|---|---|---|
| **Comments:** | | |

| 1.6.6. Repeat steps in section 1.8, 1.9, and 1.10 every 24hrs.±2hrs. until the culture is scaled up to 1 L Bioreactor culture. Typically, on day 7 of the inoculation | Operator/Date | Verifier/Date |
|---|---|---|
| Day 1 | | |
| Day 2 | | |
| Day 3 | | |
| Day 4 | | |
| Day 5 | | |
| Day 6 | | |
| Day 7 | | |
| When the suspension culture of CHO cells reaches a concentration between 5.5 X $10^6$ cells/ml and 6.5 X $10^6$ cells/ml the culture will scaled up to 1 liter Bioreactor | | |
| **Comments:** | | |

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Shake Flask ID#______________________

| **Date and time** | **TIME in culture Hrs.** | **OD 650nm** | **pH** | **Viable cells/mL** | **Percent Viability** | **GLUCOSE (g/l)** | **LACTATE (g/l)** | **Dissolved oxygen % DO** |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| Operator/verifier | Operator/verifier | Operator/Verifier | Operator/Verifier | Operator/verifier | Operator/verifier | Operator/verifier | Operator/verifier | Operator/verifier |
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| Operator/verifier | Operator/verifier | Operator/verifier | Operator/Verifier | Operator/verifier | Operator/verifier | Operator/verifier | Operator/verifier | Operator/verifier |
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| Operator/verifier | Operator/verifier | Operator/verifier | Operator/Verifier | Operator/verifier | Operator/verifier | Operator/verifier | Operator/verifier | Operator/verifier |
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| **Date and time** | **TIME in culture Hrs.** | **OD 650nm** | **pH** | **Viable cells/mL** | **Percent Viability** | **GLUCOSE (g/l)** | **LACTATE (g/l)** | **Dissolved oxygen % DO** |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| Operator/verifier | Operator/verifier | Operator/verifier | Operator/Verifier | Operator/verifier | Operator/verifier | Operator/verifier | Operator/verifier | Operator/verifier |
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|---|---|---|
| **2.0. Scale up to 1L Bioreactor**<br>When the suspension culture of NISTCHO cells reaches a concentration 5.5-7.0 x$10^6$cells/ml (typically day 6-7), the appropriate volume of culture will be used to seed 1L of NISTCHO production media in the bioreactor for an initial cell concentration of 4 x $10^5$cells/ml | | |
| **2.1. Preparation of 1M $NaHCO_3$**<br>2.1.1. Label 250 mL glass feed bottle 1MNaHCO3, [date], [initials], and storage: room temperature, disposal; drain.<br>2.1.2. Weigh out 12.6. ± 0.1 grams of (NaHCO3) sodium bicarbonate and transfer to a 250 mL beaker<br>Balance ID ________________ NaHC03 manufacturer ____________<br>Catalog number ________________ Lot number ____________<br>Expiration Date ________________<br>2.1.3. Using a 250 mL graduated cylinder, measure 145mL MilliQ water and add to the $NaHCO_3$ in the beaker<br>Volume of MilliQ water added_________ ml<br>2.1.4. Add magnetic stir bar and stir on a magnetic stirrer to dissolve. Transfer dissolved NaHCO3 to a 250 mL graduated cylinder and bring to the volume at 150 mL with MilliQ water. Transfer 150 mL 1M NaHC03 to labeled 250 ml alkaline feed bottle<br>2.1.5. Prepare labeled alkaline bottle for bioreactor - add lid and tubing per Applikon EZ-Control Bioreactor Controller Operation SOP | Operator/Date | Verifier/Date |
| **2.2. Prepare the controller as per the Applikon EZ- Control Bioreactor Controller Operator SOP step 8.1.**<br>Bioreactor ID #_____________________ | Operator/Date | Verifier/Date |
| **2.3. Assemble/Autoclave Bioreactor** | | |
| 2.3.1. Assemble the vessel stand if not already assembled.<br>2.3.2. Inspect the integrity of the large O- rings on the vessel stand and headplate. Replace if worn or cracked<br>Vessel stand: O-ring worn or cracked? Yes / No (Circle one.)<br>O-ring replaced? Yes / No (Circle one.)<br>Head plate: O-ring worn or cracked? Yes / No (Circle one.)<br>O-ring replaced? Yes / No (Circle one.)<br>2.3.3. Assemble head plate- underside.<br>2.3.3.1. Inspect the integrity of the O- rings on the sample tube, media addition tube, sparger and the thermowell.<br>Sample pipe O-ring worn or cracked? Yes / No (Circle one.)<br>O-ring replaced? Yes / No (Circle one.)<br>Media addition pipe O-ring worn or cracked? Yes / No (Circle one.)<br>O-ring replaced? Yes / No (Circle one.)<br>Sparger pipe O-ring worn or cracked? Yes / No (Circle one.)<br>O-ring replaced? Yes / No (Circle one.) | Operator/Date | Verifier/Date |

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| Thermowell pipe O-ring worn or cracked? Yes / No (Circle one.)<br>O-ring replaced? Yes / No (Circle one.)<br>2.3.3.2. Attach sample pipe, media addition pipe, sparger pipe and thermowell pipe. Verify that the sparger pipe is aligned beneath the stirrer impeller.<br>2.3.3.3. Add 100ml of 1X PBS to the bioreactor vessel<br>2.3.3.4. Attach head plate to vessel stand: Place the headplate onto the vessel stand, positioning the holes on the outer edge of the head plate with the bolts on the vessel stand and fasten with six mill nuts finger tight.<br>2.3.4. Assemble head plate – Topside.<br>2.3.4.1. Inspect the integrity of the O-ring in the condenser port of the head plate. Replaced if worn or cracked<br>Condenser port O-ring worn or cracked? Yes/No (Circle one)<br>O-ring worn or cracked? Yes / No (Circle one.)<br>O-ring replaced? Yes / No (Circle one.)<br>2.3.4.2. Inspect the black seal at the bottom of the condenser underneath the retainer nut. Replace if worn or cracked.<br>2.3.4.3. Attach the condenser to the head plate by placing the condenser into the condenser port making sure that the barbed connectors are facing out. | | |
| **2.4. Calibrate the pH sensor per SOP:Applikon Bioreactor Controller Operation (refer step 8.2.2)** | | |
| 2.4.1. Obtain pH 7 and pH 4 calibration buffers and the pH sensor.<br>2.4.2. Measure and record the temperature of the pH calibration buffers.<br>pH 7 Buffer Manufacturer:__________________<br>Catalog number: __________________<br>Lot number: __________________<br>Expiration date:__________________<br>Temperature: __________________<br>pH 4 Buffer Manufacturer:__________________<br>Catalog number: __________________<br>Lot number: __________________<br>Expiration date:__________________<br>Temperature: __________________<br>pH sensor ID: ____________<br>2.4.3. Mount pH sensor on the lab stand with clamp<br>2.4.4. Remove the protective cap from the bottom of the pH sensor. Rinse the pH sensor with de-ionized water and pat it dry with a clean lint-free laboratory wipe.<br>2.4.5. Login as Operator per section 8.1.1.3 of the "SOP: Applikon ez-Control Bioreactor Controller Operation" if not already logged in.<br>2.4.6. Remove the pH sensor screw cap. Connect the pH sensor to the pH sensor cable on the right side of the controller. Verify that | Operator/Date | Verifier/Date |

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| the pH sensor cable is plugged into the controller correctly.<br>2.4.7. Go to the controller pH Settings screen: Home > pH (bottom)<br>2.4.8. Verify that the pH control loop is off (i.e., the pH Process Value button is grey or yellow, not green). If it is on, touch the button Stop controller.<br>2.4.9. Touch the button Calibrate pH to go to the pH Calibration screen. The numerical data for Slope, Offset and any Sample correction are displayed.<br>2.4.10. Touch reset calibration value. Touch yes, when prompted are you sure?<br>2.4.11. Touch the button 2-point calibration.<br>2.4.12. Touch enter calibration value.<br>2.4.13. Enter the temperature of the buffer solutions using the numeric keypad.<br>2.4.14. When prompted for "Enter calibration first value for pH", place the pH sensor in the pH 4.0 buffer standard and wait until the shown process value stabilizes (shown near the Cancel button). Enter the pH value using the numeric keypad.<br>2.4.15. "Please wait while the stability of the pH sensor is being verified" will be displayed on the screen. After about 30 seconds The screen will display "Please enter the second calibration value" Touch "Enter Calibration"<br>2.4.16. Rinse the pH sensor and place the sensor in the pH 7.0 buffer standard. Enter the buffer temperature using the numerical keypad when prompted.<br>2.4.17. The screen will display"Enter calibration second value for pH". Using numerical Keypad enter 7.0<br>2.4.18. "Please wait while the stability of the pH sensor is being verified" will be displayed on the screen<br>2.4.19. The value for the calibrated slope and calibrated offset will be displayed<br>2.4.20. Record the slope and offset below:<br>Slope from the Display: _________ Expected value: 0.95-1.05<br>Offset from the Display: _________ Expected value: < ±0.3<br>2.4.21. If the slope value and the offset value are within specification select yes. If the slope value and offset are out of specification select no and repeat the calibration steps.<br>2.4.22. Rinse the pH sensor with DI/ MilliQ water<br>2.4.23. Disconnect the cable of the pH sensor.<br>2.4.24. Cover the pH sensor connector with the pH sensor screw cap. Verify that the rubber gasket is in place between sensor connector and the cap | | |
| **Comments** | | |

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| **2.5. Preparation and Mounting of DO sensor** | Operator/Date | Verifier/Date |
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| 2.5.1. Remove the protective cap from the bottom of the DO sensor<br>2.5.2. Inspect the screen at the bottom of the probe tip. Replace if damaged<br>2.5.3. Holding the probe in a vertical position, unscrew module from the bottom of the probe.<br>2.5.4. Inspect the integrity of the O-ring underneath the module and replace if worn or cracked.<br>2.5.5. Replenish DO electrolyte solution. There should be 1 ml of electrolyte solution in the membrane module<br>2.5.6. Screw the membrane module to the probe<br>2.5.7. Repeat step 4.6.16. to 4.6.19. for DO probe | | |

| **2.6. Preparation of the liquid addition bottles** | | |
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| 2.6.1. A process SOP should provide the information regarding the alkaline solution composition and water.<br>2.6.2. Fill a liquid 250ml addition bottle with alkaline solution, no more than 2/3 (150ml) full so that it can be autoclaved. Cap the alkaline bottle with a two-port top. If not done already<br>2.6.3. Connect the air inlet on the alkaline bottle to a gas filter using a short length (approx. 7 cm) of size 25 tubing. Do not clamp<br>2.6.4. Add 5 mL laboratory grade water to a 250 ml addition bottle to be used for transferring inoculum. This will improve the heat transfer during sterilization in the autoclave. Cap the inoculum transfer bottle with a two-port top.<br>2.6.5. Connect the air inlet on the inoculum transfer bottle to a gas filter using a short length (approx. 7 cm) of size 25 tubing.<br>2.6.6. Connect the liquid outlet on the inoculum transfer bottle to a long length (approx. 75 cm) of size 25 tubing. Attach the autoclavable male connector to the tubing with the help of cable tie<br>2.6.7. Cover the gas filters and autoclavable male connector loosely with aluminum foil.<br>2.6.8. Repeat steps 2.6. 4.to 2.8.7. with 1L media addition bottle | Operator/Date | Verifier/Date |
| **2.7. Mount Connections to the bioreactor** | | |
| 2.7.1. Connect one of the medium inlet triplet nipples to a second triplet nipple using a short length (approx. 7 cm) of size 14 tubing. Connect a medium length (approx. 15 cm) of size 14 tubing to the third medium inlet triplet nipple. Clamp the tubing closed with cable tie**.**<br>2.7.2. Connect the addition pipe to the liquid outlet on the alkaline bottle using an extra-long length (12pprox.. 75 cm) of size 25 tubing. Clamp the tubing closed with cable tie | Operator/Date | Verifier/Date |

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| 2.7.3. Connect a medium length (approx. 15 cm) of size 16 tubing with flow control tube clamp (white clamp) to the sample pipe. Attach swabable valve and cover with a piece of aluminum foil. Close the clamp.<br>2.7.4. Connect the sparger inlet to a gas filter using approx. 60 cm length of size 25 tubing. Clamp the tubing closed with cable tie<br>2.7.5. Connect a medium length (approx. 15 cm) of size 25 tubing with flow control tube clamp (white clamp) to the medium addition pipe Clamp the tubing close with white clamp. Attach a autoclavable female connector and tighten with the cable tie. Cover the female connector with aluminum foil<br>2.7.6. Connect the bottom condenser nipple on the middle condenser nipple using a medium length (approx. 15 cm) of size 25 tubing<br>2.7.7. Connect the top condenser nipple to a gas filter using approx. 50 cm of size 25 tubing. Do not clamp<br>2.7.8. Connect the top condenser nipple to a gas filter using approx. 50 cm of size 25 tubing. Do not clamp.<br>2.7.9. Insert a septum into its holder in the head plate and fasten it.<br>2.7.10. Cover the tubing ends with autoclavable aluminum foil.<br>2.7.11. Verify that the gas filter connected to the condenser (air outlet) is open to avoid pressure differences during autoclaving. Cover the gas filters loosely with aluminum foil<br>2.7.12. Verify the mountings of all nipples and other auxiliaries<br>2.7.13. Cover head plate with autoclavable aluminum foil | | |
| **2.8. Autoclave the bioreactor and liquid addition bottles** | | |
| 2.8.1. Apply autoclave indicator tape to the aluminum foil on the alkaline bottle, addition bottle and the inoculum transfer bottle.<br>2.8.2. Place the assembled bioreactor, the alkaline bottle without disconnecting tubing, in the prepared Yamato SM 501 autoclave<br>2.8.3. Place 1L addition bottle with tubing and 250ml Inoculum transfer bottle with tubing in the autoclave in the Harvey sterilmax autoclave<br>2.8.4. Loosen the caps on the alkaline bottle, liquid addition bottle and the inoculum transfer bottle.<br>2.8.5. Close the autoclave and select the sterilization temperature at 121°C for 20 minutes. (Select liquid cycle in Yamato SM 501 auotclave) for Bioreactor and select liquid cycle for Harvey sterilmax autoclave.<br>2.8.6. When the cycle completes, allow the autoclave to cool gradually. Do not open the autoclave until the temperature in the autoclave has dropped below 90°C. After reaching that temperature, open the autoclave to allow it to cool down until the contents can be unloaded safely<br>2.8.7. Tighten the caps on the alkaline bottle, inoculum transfer bottle and medium addition bottle<br>2.8.8. Remove the assembled bioreactor and the alkaline bottle together | Operator/Date | Verifier/Date |

| | | |
|---|---|---|
| and place them on the right side of the controller without disconnecting the tubing.<br>2.8.9. Remove the medium transfer bottle and inoculum transfer bottle and place it in a biological safety cabinet<br>2.8.10. Allow to cool to room temperature.<br>2.8.11. Perform a visual inspection to verify that the autoclave indicator tape changes color and that the bioreactor is dry.<br>2.8.12. Remove aluminum foil from gas filters on the bioreactor and alkaline bottle. Leave the foil on the inoculum transfer bottle and the addition bottle | | |
| **2.9. Preparation of media and media addition bottle** | | |
| 2.9.1. Obtain 1 bottle of EX-Cell Advanced CHO production medium<br>Manufacturer:_________________Catalog number: ___________________<br>Lot number:___________________Expiration date:____________________ | Operator/Date | Verifier/Date |
| 2.9.2. Obtain sterile 10 mg/mL gentamicin.<br>Manufacturer:____________________Catalog number: ____________<br>Lot number:______________________Expiration date:_____________ | Operator/Date | Verifier/Date |
| 2.9.3. Obtain sterile 45% D-glucose solution.<br>Manufacturer:____________________Catalog number: ____________<br>Lot number:______________________Expiration date:_____________ | Operator/Date | Verifier/Date |
| 2.9.4. Verify the autoclaved 1 L media addition bottle with tubing and filter with aluminum foil is in the biological safety cabinet. Do Not Remove the aluminum foil from the tubing and the filter.<br>2.9.5. Place the production media bottle, gentamicin, and D-glucose solution in the biological safety cabinet after swabbing it with 70% Ethanol<br>2.9.6. Place quantity 1 of each 100ml, 10ml and 2ml sterile individually wrapped serological pipette and pipette aid in the biological safety cabinet after swabbing it with 70% Ethanol<br>2.9.7. Using a 100ml serological pipette transfer 1L of production medium to the sterile feed bottle<br>2.9.8. Using a 10ml serological pipette, transfer 10ml of gentamicin to the feed bottle<br>2.9.9. Using a 2ml pipette transfer 1.56ml of D-Glucose solution to the feed bottle.<br>2.9.10. Aseptically transfer 10 ml of prepared medium from media addition bottle to T25 tissuse culture flask labelled “Bioreactor NISTCHO blank” “Date” “Initials” “Team Name”. Place the T25 flask in CO2 incubator<br>2.9.11. Be sure the cap is on tightly and remove the media addition bottle from the BSC and place it next to Applikon bioreactor processor | Operator/Date | Verifier/Date |
| **Comments:** | | |

| **2.10. Connecting the bioreactor to the controller** | | |
|---|---|---|
| 2.10.1. Verify that all control loops are switched off<br>2.10.2. Login as Operator per section 8.1.1.3 from the "SOP: Applikon ez-Control Bioreactor Controller Operation" if not already logged in.<br>2.10.3. Verify that all control loops are switched off. The Process Value buttons (bottom of Home Screen) should be gray or yellow. If necessary, stop controllers: Home > Menu (top right) > Start/Stop all controllers > Stop all controllers<br>2.10.4. Addition of the media to the Bioreactor<br>2.10.4.1. Carefully remove the foil from the female connector on the medium addition port of the Applikon bioreactor<br>2.10.4.2. Carefully remove the aluminum foil from the male connector on the 1L addition bottle and connect the male connector to the female connector on the medium addition port tubing of the bioreactor<br>2.10.4.3. Open the white clamp on the medium addition port of the Applikon bioreactor.<br>2.10.4.4. Remove aluminum foil from the filter attached to the medium addition bottle.<br>2.10.4.5. On the Applikon reactor touch screen select Menu >Manual Control > Acid Pump On<br>2.10.4.6. As the pump turns feed the tubing around it.Use care to avoid pinching fingers. Bend the middle of the tubing that is attached to the media addition bottle into a U shape and hold in one hand. Clip the bottom of the U into the lower pump clamp and the top of the U into the upper pump clamp, The tubing in the upper clamp should be directed to the bioreactor (Refer to figure 8 in the SOP: Applikon ez-control Bioreactor Controller Operation)<br>2.10.4.7. Once all the media has been transferred into the vessel, remove tubing from the pump head, and turn off the acid pump. On the Applikon screen select Menu > Manual control > Acid Pump off.<br>2.10.4.8. Disconnect the male connector of the addition bottle from the female connector on the sample port of the bioreactor. Close the white clamp and replace the foil<br>2.10.5. Connect the sensors<br>2.10.5.1. Remove the pH sensor screw cap. Connect the pH sensor to the pH sensor cable on the right side of the controller. Verify that the pH sensor cable is plugged into the controller correctly<br>2.10.5.2. Remove the DO sensor screw cap. Connect the DO sensor to the DO sensor cable on the right side of the controller. Verify that the DO sensor cable is plugged into the controller correctly.<br>2.10.5.3. Fill the thermometer pocket with MilliQ water in order to | Operator/Date | Verifier/Date |

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|---|---|---|
| decrease the dead time of the sensor and make temperature control more accurate. Insert the temperature sensor into the thermometer pocket. Verify that the temperature sensor cable is plugged into the controller correctly | | |
| 2.10.6. Connect the heating blanket | | |
| 2.10.6.1. Wrap the heating blanket around the bioreactor vessel (around the glass and inside the support legs). Position the blanket so that the volume markings on the vessel are visible. Fasten the blanket in place using the Velcro ends of the blanket | | |
| 2.10.6.2. Verify that the heating blanket is plugged into the controller correctly | | |
| 2.10.7. Connecting the laboratory gases air | | |
| 2.10.7.1. Connect the aeration outlet of the controller to the gas filter on the bioreactor sparger inlet using size 16 tubing. | | |
| 2.10.7.2. Open the CO2 tank and set its regulator to 20 psi. | | |
| 2.10.7.3. Turn on CO2 supply at regulator to the bioreactor.<br>Tank pressure: ______________<br>Tank output pressure: _______________ | | |
| 2.10.8. Connect the stirrer motor | | |
| 2.10.8.1. Login as Operator per section 8.1.1.3 from the “SOP: Applikon ez-Control Bioreactor Controller Operation” if not already logged in | | |
| 2.10.8.2. Go to the Stirrer Settings screen: Home > Stirrer (bottom left) | | |
| 2.10.8.3. Touch limit button >Lower limit > enter 58 | | |
| 2.10.8.4. Touch stirrer settings >enter 60 for setpoint | | |
| 2.10.8.5. Start the stirrer: touch the button Start controller | | |
| 2.10.8.6. Position the stirrer motor vertically over the bioreactor head plate and slowly lower it into place. Verify that the impeller is turning | | |
| 2.10.8.7. Stop the stirrer: touch the button Start Stirrer controller | | |
| 2.10.9. Connect the alkaline bottle | | |
| 2.10.9.1. Login as Operator per section 8.1.1.3 from the “SOP: Applikon ez-Control Bioreactor Controller Operation” if not already logged in. | | |
| 2.10.9.2. Cut the zip tie | | |
| 2.10.9.3. Locate the alkaline pump on the right front panel of the controller. Open the pump cover. | | |
| 2.10.9.4. Turn the pump on manually: Home > Menu > Manual control>Alkaline pump: On | | |
| 2.10.9.5. As the pump turns feed the tubing around it.Use care to avoid pinching fingers. Bend the middle of the tubing that is attached to the alkaline bottle into a U shape and hold in one hand. Clip the bottom of the U into the lower pump clamp and the top of the U into the upper pump clamp, The tubing in the upper clamp should be directed to the bioreactor. | | |
| 2.10.9.6. Watch the solution being drawn from the bottle into the tubing. | | |

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| When the solution reaches 3 inches from entering the bioreactor, turn the pump off. | | |
| **2.11. Enter Process parameter setting** | | |
| 2.11.1. Login as Operator per section 8.1.1.3 from the "SOP: Applikon ez-Control Bioreactor Controller Operation" if not already logged in.<br>2.11.2. Go to the pH Settings screen: Home > pH (bottom)<br>2.11.3. Enter the process settings for the pH control loop:<br>Enter the pH upper limit: pH settings > limits > High limit >Enter 7.20.<br>Enter the pH lower limit: pH settings > limits > Low limit>Enter 7.10.<br>Enter the pH setpoint > setpoint>Enter 7.15.<br>2.11.4. Go to the temperature setting screen: Home>Temperature<br>Enter the process settings for temperature control loop:<br>Enter temperature upper limit: temperature setting>Limits> High limit>Enter 38<br>Enter temperature lower limit:>Limits>Low limit>Enter 36<br>Enter temperature setpoint>setpoint>Enter 37<br>2.11.5. Go to stirrer setting screen: Home>Stirrer<br>Enter the process settings for stirrer control loop:<br>Enter stirrer upper limit: stirrer setting>limits>High limit>enter 151<br>Enter stirrer lower limit: limits>low limit>enter 149<br>Enter stirrer setpoint: stirrer settings>setpoint> enter 150 | Operator/Date | Verifier/Date |
| **2.12. Start the controller loop for temperature, stirrer and pH. Don't start the DO control loop** | | |
| 2.12.1. Login as Operator per section 8.1.1.3 from the "SOP: Applikon ez-Control Bioreactor Controller Operation" if not already logged in<br>2.12.2. Start temp control loop: Home > temp (bottom) >Start temp loop<br>2.12.3. Start Stirrer control loop: Home> stirrer (bottom)> Start stirrer loop<br>2.12.4. Once the media temperature reached 37 °C start the pH control loop: Home > pH (bottom)>Start pH control loop<br>2.12.5. Allow the process to run for at least 6 hours | Operator/Date | Verifier/Date |
| **2.13. Media Hold and DO probe Polarization.**<br>**Perform Media hold and DO probe polarization simultaneously. Media should be held for 24 hrs. to check for contamination and DO probe should be polarized for at least 6 hours before calibration.** | | |
| 2.13.1. Check media for contamination after 24 hrs. ± 2hrs.<br>Incubation start time: _______________<br>Incubation end time: _______________<br>Elapsed time: _______________<br>Contamination? Yes / No (Circle one.) | Operator/Date | Verifier/Date |
| **Comments:** | Operator/Date | Verifier/Date |

| 2.13.2. Calibrate the DO sensor<br>2.13.2.1. Open the Air tank. Set the output pressure at 30 psi. Record the Air tank pressure: _____psi and output pressure:____ psi<br>2.13.2.2. Login as Operator per section 8.1.1.3 from the "SOP: Applikon ez-Control Bioreactor Controller Operation" if not already logged in<br>2.13.2.3. Verify that the medium in the bioreactor is stable at process temperature.<br>2.13.2.4. Go to DO control loop>Home> touch dO2<br>2.13.2.5. Verify if the measuring range is set to Air: dO2 settings>sensor settings>Air should be highlighted.<br>2.13.2.6. Reset calibration values: dO2 settings>calibrate>reset calibration values>Select yes when prompted "Are you sure you want to reset the calibration values"<br>2.13.2.7. Open aeration valve manually: dO2settings>Manual Control>O2 valve>On<br>2.13.2.8. Continue aeration until dO2 reading is stable (15 to 20 minutes)<br>2.13.2.9. Close the aeration valve manually: Home>dO2>Manual Control>O2 valve>Off<br>2.13.2.10. Calibrate the dO2 sensor: dO2 settings>calibrate>1 point calibration>Enter Calibration Value> Enter 100<br>2.13.2.11. The screen will display "Please wait while the stability of the dO2 sensor is being verified<br>2.13.2.12. The screen will display "Calibrated slope is:______and calibrated offset is_____<br>Do you want to apply this slope and offset?<br>Yes No<br>2.13.2.13. Verify the calibrated slope value is within the specification:<br>2.0 to 4.0 at 25°C<br>1.5 to 3.0 at 37°C<br>2.13.2.14. If the calibrated slope is within the specification enter yes. If not within the specifications enter No and repeat the calibration steps<br>2.13.2.15. Enter the process settings for dO2 control loop:<br>Home>dO2 settings>limits>high limit>enter 41<br>dO2 settings>limits>Low limit>enter 39<br>dO2 settings>setpoint>enter 40<br>2.13.2.16. Start the dO2 control loop: dO2 settings> Start Controller | Operator/date | Verifier/Date |
|---|---|---|
| **2.14. Reset dose monitor values**<br>**When all control loops are at set-point, the bioreactor system is ready for cultivation. All Dose Monitor values should be reset to 0ml** | | |
| 2.14.1. Home>Menu>Dose Monitor>Reset all dose monitors>Are you sure?> Yes | Operator/Date | Verifier/Date |
| **Comments** | Operator/Date | Verifier/Date |
| **3.0. Starting the cultivation** | | |

| **3.1.** Inoculate the bioreactor. | Operator/Date | Verifier/Date |
|---|---|---|
| 3.1.1. Place the autoclaved inoculum transfer bottle in the BSC if not done already<br>3.1.2. Do not remove the foil from the gas filter or the autoclavable male connector Transfer the appropriate amount of cell suspension from the shake flask to the inoculum bottle. Refer to the process SOP for the detail of the number of cells required to inoculate the bioreactor. Recap the bottle before removing it from the biosafety cabinet. Place the inoculum bottle on the right side of the controller<br>Volume of inoculum transferred in the feed bottle: _______ml<br>3.1.3. Login as Operator per section 8.1.1.3. from the "SOP: Applikon ez-Control Bioreactor Controller Operation" if not already logged in<br>3.1.4. Stop all control loops: Home > Menu > Start/Stop all controllers > Stop all controllers<br>3.1.5. Swab gloves with 70% isopropanol; spray the female connector on the medium addition port<br>3.1.6. Remove the foil from the attached tubing connected to the male connector and the gas filter on the inoculum transfer bottle. Connect the female connector on the sample port of the bioreactor to the male connector of the inoculum bottle<br>3.1.7. Open the white clamp located on the medium addition port of the Applikon bioreactor<br>3.1.8. Start the acid pump: Home>Menu>Manual Control>Acid pump>On<br>3.1.9. As the pump turns feed the tubing around it. Use care to avoid pinching fingers. Bend the middle of the tubing that is attached to the inoculum bottle into a U shape and hold in one hand. Clip the bottom of the U into the lower pump clamp and the top of the U into the upper pump clamp, The tubing in the upper clamp should be directed to the bioreactor<br>3.1.10. Once all of the cell suspension has been transferred into the vessel stop the acid pump: Home>Menu>Manual Control>Acid pump>off<br>3.1.11. Close the white clamp located on the medium addition port. Remove the tubing from the pump head.<br>3.1.12. Disconnect the male connector of the addition bottle from the female connector on the sample port of the bioreactor. Cover the female connector with the aluminum foil<br>3.1.13. Re-start all control loops: Home > Menu > Start/Stop all controllers > Start all controllers<br>3.1.14. 15 minutes after inoculation (Day0) and at 24 hrs. intervals, sample the culture to determine OD, viable cell count, cell viability, glucose and lactate concentration. Refer to step 3.2. | | |
| **Comments:** | Operator/Date | Verifier/Date |

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| **3.2.** Monitoring the cell culture<br>The cell culture is sampled every 24 hrs. ± 2 hrs.in order to measure cell concentration and perform product assays per the process SOP until the cell concentration reaches between 4-5 X $10^6$cells/ml typically day 6. At this cell concentration, the conditioned media in the bioreactor is harvested. | Operator/Date | Verifier/Date |
|---|---|---|
| Day 0 | | |
| Day 1 | | |
| Day 2 | | |
| Day 3 | | |
| Day 4 | | |
| Day 5 | | |
| Day 6 | | |
| Day 7 | | |
| Day 8 | | |
| Day 9 | | |
| Day 10 | | |
| 3.2.1. Sampling procedure:<br>Collect :<br>• Sterile 10 ml syringe quantity 2<br>• Sterile 15 ml conical tube labelled with team initial, date<br>• 0.2 µ sterile syringe filter connected to a 10ml sterile syringe<br>• 2-Spectrophotometer cuvettes as “blank” and “sample”<br>• Sterile 1.5ml microfuge tube labelled glucose/lactate, day, team initials<br>• Sterile 1.5ml microfuge tube labelled cell count<br>• Sterile 1.5ml microfuge tube labelled “Blank”<br>• 50ml beaker labelled waste<br>• Spray bottle with 70% EtOH<br>3.2.2. In the BSC, aseptically transfer 1.1 ml of the blank solution from the T25 tissue culture flask labelled “Bioreactor NISTCHO blank” to sterile microfuge tube labelled “blank”<br>3.2.3. Login as Operator per section 8.1.1.3 from the “SOP: Applikon ez-Control Bioreactor Controller Operation” if not already logged in.<br>3.2.4. Spray the sampling tube end cap with 70% ethanol.<br>3.2.5. Unscrew the end cap on the sample port tubing<br>3.2.6. Connect a sterile 10ml syringe to the female lure lock on the sample port tubing.<br>3.2.7. Open the white clamp such that it allows the liquid to flow<br>3.2.8. Draw 5ml culture into a sterile 10ml syringe to prime the line, dispose of culture in a waste beaker to be bleached<br>3.2.9. Connect a sterile 10ml syringe to the female lure lock on the sample port tubing. | Operator/Date | Verifier/Date |

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| 3.2.10. Draw 5ml of culture for test sample. Transfer the culture into 15ml conical tube<br>3.2.11. Disconnect the sample port tubing from the syringe.<br>3.2.12. With a 10ml syringe connected to a 0.2µ filter unit push clean air into the sample port to expel culture in the sample pipe back into a vessel<br>3.2.13. Close the white clamp and disconnect the filter unit connected to a syringe.<br>3.2.14. Use this sample to perform cell viability assay, OD 650 measurement, and Glucose and Lactate concentration measurement.<br>3.2.15. Cell concentration and viability determination:<br>3.2.15.1. Mix the bioreactor sample in the 15ml conical tube by gently inverting the tubes 5 times. Transfer 100µl of cell suspension to the sterile 1.5ml microfuge tube labelled "cell count"<br>3.2.15.2. Using the 100 µl of cell suspension from microfuge tube labelled "cell count" from the step above determine cell count and cell viability using "Operation of Logos Biosystems Luna-FL Fluorescence Cell Counter for Fluorescence Cell Counting SOP"<br>3.2.15.3. Record the viable cell count cells/ml and % viability in the Bioreactor data table<br>3.2.16. OD Measurement at 650 nm:<br>Collect<br>• Two cuvettes and a cuvette holder<br>• P1000 micropipette and tips<br>• Microfuge tube labelled G/L and initials<br>3.2.16.1. Turn on the Genesys 180 spectrophotometer 5 minutes before measuring the absorbance.<br>3.2.16.2. The main menu will be displayed once the power on is completed (about 2 to 3 minutes)<br>3.2.16.3. Select the "Fixed" icon on the main screen.<br>3.2.16.4. Select the "+" icon located in the bottom right corner to create a new protocol.<br>3.2.16.5. Change the method name to "vessel name, team name and date" and press done.<br>3.2.16.6. Press "SETUP" located on the top right corner of the screen<br>3.2.16.7. Select "8 cell changer" and enter "1" for the number of cell changer.<br>3.2.16.8. Change the wavelength to 650nm by pressing the number displayed and enter 650<br>3.2.16.9. Unselect 8-3 and select the "←" in the top left to return to the SETUP screen.<br>3.2.16.10. Open the sample compartment lid. Align the white arrow on the 8-cell changer with the arrow on the sample compartment by manually turning the changer with the blue knob. | | |

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| 3.2.16.11. Label one cuvette "B" and one cuvette "S" (Note: Don't touch the cuvette below the frosted area<br>3.2.16.12. Transfer 1 ml of blank to the cuvette labelled "B" and 1 ml of sample to the cuvette labelled "S" using p1000 micropipette (Note: mix the sample by pipetting up and down gently before transferring to the cuvette to take a representative sample)<br>3.2.16.13. Place the cuvette labelled blank in sample holder 1 and cuvette labelled sample in sample holder 2. Load the cuvettes such that the longer path length (10mm) is perpendicular to the white arrow of the sample compartment. Close the sample compartment lid.<br>3.2.16.14. Press "continue" on the display screen.<br>3.2.16.15. Record the measured absorbance in the data table for the shake flask.<br>3.2.16.16. Select three dots icon on the top right of the screen. Select print icon on the screen to print the results.<br>3.2.16.17. When done exit the menu by pressing "X" on the top left side of the screen. Press "end experiment".<br>3.2.16.18. Remove cuvettes from the spectrophotometer.<br>3.2.16.19. Transfer 1ml of sample from the 15ml conical tube to a 1.5 ml sterile microfuge tube labelled "G/L" and team name. Centrifuge the tube for 5 minutes in the microcentrifuge.<br>3.2.16.20. Transfer 980µl of the supernatant to a new sterile 1.5 ml microfuge tube. Label the tube with day of the culture, date, type of vessel (shaker or bioreactor) and team initials.<br>3.2.16.21. Add bleach to the tube with cell pellet and sample cuvette and discard in the biohazard waste. Drain the blank solution in the sink and discard the blank cuvette in the biohazard waste.<br>3.2.17. Glucose and Lactate Measurement<br>3.2.17.1. Collect Microfuge tube labelled G/L from step 5.4.20.<br>3.2.17.2. Measure Glucose and Lactate concentration using the YSI Bioanalyzer by following "SOP Measurement of Glucose and Lactate concentration in Media using YSI 2500 Bioanalyzer<br>3.2.17.3. Following glucose/lactate determination record the data in the shake flask table. Store the sample tube at 2-8°C in a microfuge storage box labeled with your team's name | | |
| **Comments** | Operator/Date | Verifier/Date |

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| **4.0.End of Run- Culture Harvest**<br>**When the cell culture reaches the plateau or early decline phase (typically between day 7-10 of the run), the culture media is harvested** | | |
|---|---|---|
| **4.1.** Stop the controllers<br>4.1.1. Login as Operator per section 8.1.1.3. from the “SOP: Applikon ez-Control Bioreactor Controller Operation” if not already logged in<br>4.1.2. Stop all control loops: Home > Menu > Start/Stop all controllers > Stop all controllers.<br>4.1.3. Close the CO2 tank.<br>4.1.4. Close the air tank<br>**4.2.** Disconnect the bioreactor<br>4.2.1. Locate the tubing that connects the alkaline bottle to the bioreactor. Clamp the tubing near the bioreactor. Disconnect the tubing from the alkaline bottle and remove the tubing from the controller pump<br>4.2.2. Lift the stirrer motor from the bioreactor head plate and set the motor aside.<br>4.2.3. Disconnect the gas filter on the bioreactor sparger inlet from the tubing to the aeration outlet of the controller.<br>4.2.4. Unwrap the heating blanket from around the bioreactor vessel and set the blanket aside being sure it is lying flat.<br>4.2.5. Disconnect the pH sensor cable from the pH sensor. Cover the pH sensor connector with the pH sensor screw cap<br>4.2.6. Repeat step 4.2.5. for the DO sensor<br>**4.3.** Cleaning the pH and DO sensor<br>4.3.1. Remove pH and DO sensors from the bioreactor head plate<br>4.3.2. Rinse the pH and DO sensors thoroughly with MilliQ water, being careful to remove all broth-residue. Gently pat dry with a clean lint-free laboratory wipe. Spray with 70% IPA and gently pat dry with a clean lint-free laboratory wipe<br>4.3.3. Rinse with MilliQ water and pat dry with a clean lint-free laboratory wipe.<br>4.3.4. Fill the protective cap of the pH sensor 1/2 full of 3M potassium chloride (KCl/pH probe storage solution) solution. Cover the tip of the pH sensor with its protective cap. Verify that the pH electrode is completely immersed in KCl solution<br>4.3.5. Cover the tip of the DO sensor with its protective cap. The DO sensor can be stored in an electrolyte solution upright for a short term. For long term storage, store dry after discarding the electrolyte solution<br>**4.4.** Transfer the cell culture<br>4.4.1. Loosen the six mill nuts that fasten the head plate on the bioreactor vessel and remove the head plate and place in a bin<br>4.4.2. Transfer the 200ml cell culture into 250 ml autoclaved centrifuge bottles by pipetting with 100ml serological pipette. Record the | Operator/Date | Verifier/Date |

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| volume of the cell culture. Use more centrifuges bottles as required to transfer all the culture from the bioreactor vessel<br>Volume of culture harvested: _________ml<br>4.4.3. Weigh and match the weight of the 2 centrifuge bottles in order to balance the weight in the centrifuge. Repeat with rest of the centrifuge bottles.<br>4.4.4. Centrifuge cells in pre-chilled Sorvall centrifuge, fitted with a SLA1500 rotor, at 2500 x g for 10 min at 4°C<br>4.4.5. Transfer supernatant from centrifuge bottles to sterile storage bottle by pipetting the supernatant, being careful not to disturb the pellet. Record the volume of conditioned medium transferred: ______ml<br>4.4.6. Sterile filter the conditioned media using the 0.2µm filter unit. Store the filtered condition media in the appropriately labeled storage bottle 4°C for short term and at -20°C for long term. Add protease inhibitors and 10% tween 80 before storage: Add appropriate volume of 100X protease inhibitor cocktail to generate a 1X final concentration. Total volume of 100X protease inhibitor cocktail added: _______<br>Add 2.5ml of 10% tween 80 to 250ml of clarified medium total volume of 10% tween 80 added<br>4.4.7. The clarified medium will be used for downstream processing.<br>4.4.8. Re-mount the head plate on top of the vessel and fasten with the six mill nuts finger-tight | | |
| **5.0.Bioreactor Shut down and Storage** | | |
| **5.1. Bioreactor clean in place**<br>5.1.1. Loosen the six mill nuts that fasten the head plate on the bioreactor vessel and remove the head plate<br>5.1.2. Fill the bioreactor with a working volume of 0.1M NaOH solution (2.4 liters for a 3-liter bioreactor).<br>5.1.3. Re-mount the head plate on top of the vessel and fasten with the six mill nuts finger-tight<br>5.1.4. Connect the stirrer motor per section 2.10.8.<br>5.1.5. Change the stirrer high limit to 251 rpm and setpoint to 250rpm.<br>5.1.6. Activate the stirrer at 250 RPM for 30 minutes. Visual check for dissolution of foam, debris, and other contamination in the bioreactor<br>5.1.7. Stop the stirrer. Lift the stirrer motor from the bioreactor head plate and set the motor aside.<br>5.1.8. Loosen the six mill nuts that fasten the head plate on the bioreactor vessel and remove the head plate<br>5.1.9. Drain the bioreactor<br>**5.2. Disassemble the bioreactor and clean all parts.**<br>5.2.1. Remove all the tubing and gas filters from the bioreactor head plate assembly<br>5.2.2. Remove septum from the head plate | Operator/Date | Verifier/Date |

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|---|---|---|
| 5.2.3. Remove the air outlet condenser from the head plate and disassemble the condenser for cleaning<br>5.2.4. Remove the head plate from the bioreactor vessel<br>5.2.5. Clean all parts carefully and thoroughly using a small soft bristle brush and a dilute laboratory glassware cleaner. Rinse thoroughly with milliQ water and spray with 70% EtOH/IPA and place on paper towels on a lab bench to dry.<br>5.2.6. Let dry all the parts<br>**5.3. Clean the porous sparger tip (optional)**<br>5.3.1. Remove the sparger tip from the air inlet pipe if not done already<br>5.3.2. Soak the sparger overnight in a solution of 10 mg/mL pepsin / 0.01M HCl.<br>5.3.3. Use ultrasonic cleaning with water and/or ethanol<br>5.3.4. Replace the sparger tip onto the air inlet pipe | | |
| **6.0. Prepare the growth curve for spinner flask samples and Bioreactor samples.**<br>**Shake Flask**<br>Cells/mL, and glucose vs. time (use 2 y-axis)<br>Cells/ml, and lactate vs. time (use 2 y-axis)<br>Cells/ml, % viability vs. time (use 2 y-axis)<br>Cells/ml, OD 650nm vs. time (use 2 y-axis)<br>Cells/ml, pH vs. time (use 2 y-axis)<br>Glucose and lactate vs. time (use 2 y axis)<br>Attach graphs to Batch Record**.**<br>**Bioreactor**<br>Cells/mL, and glucose vs. time (use 2 y-axis)<br>Cells/ml, and lactate vs. time (use 2 y-axis)<br>Cells/ml, % viability vs. time (use 2 y-axis)<br>Cells/ml, OD 650nm vs. time (use 2 y-axis)<br>Cells/ml, pH vs. time (use 2 y-axis)<br>Glucose and lactate vs. time (use 2 y axis)<br>Attach graphs to Batch Record. | Operator/Date | Verifier/Date |
| **Comments:** | | |

**Batch Record: Batch Culture of NISTCHO Cells for Production of cNISTmAb**
**Lot Number ___________________________**

**Applikon Bioreactor ID #___________________________**

| **Date and time** | **TIME in culture Hrs.** | **pH** | **OD 650nm** | **Viable cells/mL** | **Percent Viability** | **GLUCOSE (g/l)** | **LACTATE (g/l)** | **Dissolved oxygen % DO** |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| 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: Batch Culture of NISTCHO Cells for Production of cNISTmAb**
**Lot Number ___________________________**

| **Date and time** | **TIME in culture Hrs.** | **pH** | **OD 650nm** | **Viable cells/mL** | **Percent Viability** | **GLUCOSE (g/l)** | **LACTATE (g/l)** | **Dissolved oxygen % DO** |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| Operator/verifier | Operator/verifier | Operator/verifier | Operator/verifier | Operator/verifier | Operator/verifier | Operator/verifier | Operator/verifier | Operator/verifier |
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