SOP: Batch Culture of NISTCHO Cells for Production of cNISTmAb

Approvals:

Preparer: Hetal Doshi and Dr. Maggie Bryans Date: 29DEC23
Reviewer: Shanna Milligan Date: 10JAN24

1. **Purpose:**
   1.1. Batch culture of the NISTCHO cell line to produce recombinant anti RSV cNIST monoclonal antibody (mAb). Cells will be cultured in a 100ml shake flask culture and scaled up to a 1L culture in a bioreactor.
2. **Scope:** Applies to the production of recombinant cNIST monoclonal antibodies from the recombinant Chinese Hamster Ovary (CHO) cell line NISTCHO.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1 NISTCHO Test Material, Clonal CHO-K1 Cell Line Producing cNISTmAb Guidance Document https://tsapps.nist.gov/srmext/certificates/10197.pdf
   4.1. SOP: Labconco Purifier Class 2 Biological Safety Cabinet Operation, Document No. UP 1
   4.2. SOP: Oakton PC 700 Bench Series pH/Conductivity/°C/°F Meter, Document No MET1
   4.3. SOP: Operation of Logos biosystems Luna-FL Fluorescence Cell Counter for Fluorescence Cell Counting
   4.4. SOP: Applikon EZ-Control Bioreactor Controller Operation, Document No. UP 4
5. **Precautions:**
   5.1. Use BL2 safety measures and discard waste in biohazard containers.
   5.2. Routine care should be exercised in the handling of buffers and samples of biological materials, which may have harmful biological activity in the case of accidental ingestion, needle stick etc.
   5.3. Gloves, a lab coat and protective eyewear should be worn when handling buffers and samples.
6. **Equipment and Materials:**
   6.1. Equipment
      6.1.1. Biological safety cabinet
      6.1.2. CO_2 incubator with shaking platform
      6.1.3. 250ml Shake Flask, Erlenmeyer, PETG, with HDPE Vent Cap, Sterile, Chemglass, CGN-2092-250
      6.1.4. Fisher Scientific Isotemp Low speed magnetic stirrer
      6.1.5. Thermo Scientific Evolution 220 UV-vis spectrophotometer
      6.1.6. Oakton PC 700 Bench Series pH/Conductivity/°C/°F Meter/ micro pH probe
      6.1.7. Fisherbrand microcentrifuge
      6.1.8. Luna-FL Fluorescence cell counter
      6.1.9. Applikon EZ-control bioreactor controller with A 3-liter glass autoclave bioreactor and the processor
      6.1.10. 500 ml and 1L liquid addition/feed bottles
      6.1.11. 250 ml glass feed bottle for 150 ml alkaline solution

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6.1.12. 100 ml glass bottle
6.1.13. Sterile sample bottle with autoclavable silicone tubing size 16 about 12 cm in length
6.1.14. Male and female autoclave connectors
6.1.15. Tubing clamps
6.1.16. Gas filters, 0.2µm
6.1.17. Autoclavable silicone tubing size 14(1.6mm interior diameter)
6.1.18. Autoclavable silicone tubing size 16(3.1mm interior diameter)
6.1.19. Autoclavable silicone tubing size 25(4.8mm interior diameter)
6.1.20. Laboratory gasses: Air compressor, CO_2, O_2(optional)
6.1.21. YSI 2500 Biochemistry Analyzer
6.1.22. Micro combination pH Electrode, 9810BN

6.2. Materials:

6.2.1. Vials of NISTCHO cells (RGTM 10197)
6.2.2. EX-CELL® CD CHO Fusion Media catalog number: 14365C-1000ML (Will be referred to as expansion media)
6.2.3. Ex- Cell Advanced CHO Fed- Batch Media catalog number: 14366C-1000ML (Will be referred to as production media)
6.2.4. Nalagene 500 ml 0.2 µm filter units
6.2.5. 1X PBS
6.2.6. 150 ml of 1M $NaHCO_3$ (sodium bicarbonate)
6.2.7. 10mg/ml gentamycin
6.2.8. 45% D (+)-Glucose solution
6.2.9. Ex-CELL antifoam (Sigma, 59920C-1B)
6.2.10. O_2 Electrolyte solution for DO probe
6.2.11. 100ml and 250 ml graduated cylinder
6.2.12. Sterile serological pipettes (2ml, 5ml, 25 ml, and 50 ml)
6.2.13. Pipette aid
6.2.14. Spectrophotometer UV/Vis cuvettes and cuvette rack
6.2.15. Oakton pH 4.0 and pH 7.0 standard buffers
6.2.16. 50 ml beakers
6.2.17. 1-T25 vented tissue culture flask for blank
6.2.18. Test tube rack
6.2.19. 1.5 ml microfuge tube and tube holder
6.2.20. P20, P200, and P1000 micropipettes and compatible tips
6.2.21. Sterile 250ml glass bottles for storage of CHO cell media
6.2.22. Sterile sample bottles with size 16 about 12 cm length
6.2.23. Aluminum foil
6.2.24. Autoclave tape
6.2.25. Cotton
6.2.26. YSI 2363 Potassium Ferrocyanide (FCN)
6.2.27. YSI 1531 D-Glucose standard 9.00 g/L
6.2.28. YSI 1530 L-Lactate standard 30.0 mmol/L

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6.2.29. 1.5 mL microfuge tubes and tube rack
6.2.30. Cell culture media samples.
6.2.31. P-1000 micropipette and tips

7. Procedure:

The batch record should be completed step by step by operator of the task and the verifier of the task.

7.1. Preparation of shake flasks and Blank

7.1.1. Prepare BSC according to SOP
7.1.2. Obtain a 250ml shake flask and place in prepared BSC.
7.1.3. Aseptically transfer 98ml of growth media to the shake flask
7.1.4. Aseptically transfer 20 ml of growth media to a T25 vented tissue culture flask, this media will be used to blank the spectrophotometer for OD testing
7.1.5. Label the shake flask as NISTCHO, [date], [team name]. Label the T25 tissue culture flask as BLANK, [date], [team name].
7.1.6. Place the shake flask in the CO_2 incubator. Set the shaking speed to 125rpm.
7.1.7. Place T25 tissue culture flask containing the growth media in the CO_2 incubator.
7.1.8. Verify that the temperature is 37 ± 0.5°C and the percentage of CO_2 is 5 ±0.5% and the shaking speed is 125rpm
7.1.9. Check media for contamination after a minimum of 24 hours.

7.2. Inoculation of shake flask

7.2.1. Prepare a biological safety cabinet per SOP.
7.2.2. Remove two vials of NISTCHO cells from storage in the -150°C freezer and record removal of the two vials in the -150°C freezer log. **Each vial should contain 1.3 x 10^7 cells in 1ml to obtain an initial culture concentration of 2.6 x 10^5cells/ml after inoculation.**
7.2.3. Thaw vial contents rapidly using the Thawstar device one at a time with the non thawing vial store in the pre chilled cryo transporter
7.2.4. Spray vials with 70% isopropanol/EtOH and place them in the biological safety cabinet.
7.2.5. Quickly remove the shake flask from the incubator spray and place it in the BSC for inoculation.
7.2.6. Aseptically transfer the entire contents of both 1 ml vials of thawed NISTCHO cells into the shake flask. Do not add cells to the T25 tissue culture flask labelled BLANK
7.2.7. Swirl to mix. Place the shake flask back in the incubator set at 37°C with 5% CO_2 and 125rpm for 15 minutes.
7.2.8. Take a day 0 sample following the procedure described in 7.3.

7.3. Monitoring/Sampling the cell culture

In the BSC remove a 1.2ml sample of the culture 15 minutes after inoculation. This will be the day 0 sample. This step will be repeated at specified time points to monitor cell growth and viability and culture conditions. Analyze samples from each time point using tests for: (1) optical density at 650 nm, (2) viable cell density (3) glucose/lactate concentration (4) pH. Samples will be tested every 24 hrs. + 2 hrs. until day 7 (cell density should be 5.5-6.5 x10^6 cells/ml), the culture can then be used to inoculate the bioreactor.

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7.3.1. Sampling the culture:

7.3.1.1. Prepare biological safety cabinet per SOP

7.3.1.2. Collect the following items, spray with 70% EtOH and place in Biological Safety Cabinet:

- 1.5ml Microfuge tube labelled NISTCHO
- 1.5ml microfuge tube labelled blank
- microfuge tube holder
- 1-Pipette aid
- 2, 2ml individually wrapped serological pipette

7.3.1.3. Remove BLANK T25 tissue culture flask from CO_2 incubator, spray 70% IPA and place in BSC

7.3.1.4. Using aseptic technique, remove 1.1 mL from the BLANK T25 flask and place into a 1.5 mL microfuge tube labeled blank

7.3.1.5. Remove shake flask labelled NISTCHO, spay and place in BSC

7.3.1.6. 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.

7.3.1.7. Return NISTCHO flask and BLANK T25 tissue culture flask to the CO_2 incubator

7.3.1.8. Mix the 1.2 mL cell suspension by inverting the 1.5 mL tube several times. On the benchtop transfer 100 µl of cell suspension to the tube labelled "cell count"

7.3.1.9. **Cell concentration and viability determination**

7.3.1.9.1. 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"

7.3.1.9.2. Record all data in the production batch record

7.3.1.10. **pH measurement**

7.3.1.10.1. Calibrate the pH meter with microprobe according to SOP

7.3.1.10.2. Place the microprobe into the 1.5ml tube containing 1.1ml of culture

7.3.1.10.3. Measure and record pH

7.3.1.10.4. Rinse probe with Milli Q water, 70% EtOH, followed by MilliQ water

7.3.1.11. **OD Measurement at 650nm**

Collect

- Two cuvettes and a cuvette holder
- P1000 micropipette and tips
- Microfuge tube labelled G/L and initials

7.3.1.11.1. Turn on the Genesys 180 spectrophotometer 5 minutes before measuring the absorbance.

7.3.1.11.2. The main menu will be displayed once the power on is completed (about 2 to 3 minutes)

7.3.1.11.3. Select the "Fixed" icon on the main screen.

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7.3.1.11.4. Select the "+" icon located in the bottom right corner to create a new protocol.
7.3.1.11.5. Press "SETUP" located on the top right corner of the screen.
7.3.1.11.6. Select "8 cell changer" and enter "1" for the number of cell changer.
7.3.1.11.7. Unselect 8-3 and select the "←" in the top left to return to the SETUP screen.
7.3.1.11.8. Change the wavelength to 650nm by pressing number displayed and enter 650.
7.3.1.11.9. Open the sample compartment lid. Align white arrow on the 8-cell changer with the arrow on the sample compartment by manually turning the changer with the blue knob.
7.3.1.11.10. Label one cuvette "B" and one cuvette "S" (Note: Don't touch the cuvette below the frosted area)
7.3.1.11.11. 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)
7.3.1.11.12. 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.
7.3.1.11.13. Press "continue" on the display screen. Press start.
7.3.1.11.14. Record the measured absorbance in the batch record.
7.3.1.11.15. Select three dots icon on the top right of the screen. Select print icon on the screen to print the results.
7.3.1.11.16. When done exit the menu by pressing "X" on the to left side of the screen. Press "end experiment".
7.3.1.11.17. Remove cuvettes from the spectrophotometer.
7.3.1.11.18. 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.
7.3.1.11.19. 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
7.3.1.11.20. 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.

7.3.1.12. **Glucose and Lactate Measurement**

Collect:

- Microfuge tube labelled G/L from step 7.3.1.11.19

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7.3.1.12.1. Measure Glucose and Lactate concentration using the YSI Bioanalyzer by following "SOP Measurement of Glucose and Lactate concentration in Media using YSI 2500 Bioanalyzer

7.3.1.12.2. Following glucose/lactate determination store the sample tube at 2-8°C in a microfuge storage box labeled with team name

7.4. Scale up to 1L bioreactor

Note: 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.

7.4.1. Prepare and autoclave addition/feed bottles

7.4.1.1. Prepare 250 mL alkaline bottle containing 150 mL of 1M NaHCO3 in deionized water

7.4.1.1.1. Weigh out 12.6 ± 0.1g of NaHCO3 and transfer to a 250 mL beaker.

7.4.1.1.2. Using a 250 mL graduated cylinder, measure 145 mL MilliQ water and add to the 12.6 ± 0.1g of NaHCO3 in the beaker. Add a magnetic stir bar and stir on a magnetic stirrer to dissolve.

7.4.1.1.3. Transfer dissolved 1 M NaHCO3 solution to 250 mL graduated cylinder and bring to 150 mL volume with MilliQ water.

7.4.1.1.4. Label the bottle as 1M NaHCO3, [date], [initials], [group number], storage: room temp, disposal: drain.

7.4.1.1.5. Transfer 150 mL solution to the labeled 250 mL alkaline feed bottle

7.4.1.1.6. Prepare labeled alkaline bottle for bioreactor - add lid and tubing per Applikon EZ-Control Bioreactor Controller Operation SOP.

7.4.1.2. Add 100 mL of 1X PBS to the Bioreactor vessel. (NOTE: Do not autoclave the bioreactor with the media

7.4.1.3. Prepare 1L addition bottle with tubing and autoclavable male connector attached for autoclaving per Applikon EZ-Control Bioreactor Controller Operation SOP, steps 8.2.4).

7.4.1.4. Prepare the Applikon bioreactor with attached alkaline bottle for autoclaving per Applikon EZ-Control Bioreactor Controller Operation SOP (steps described in 8.2 and 8.3)

7.4.1.5. Autoclave the Applikon bioreactor containing 100 mL of 1X PBS with an alkaline bottle attached. Autoclave 1L addition bottle with tubing and autoclavable male connector attached per the Applikon ez-Control Bioreactor Controller Operation SOP (step 8.3)

7.4.2. Prepare bioreactor for cultivation

To prepare the bioreactor for cultivation, 1L of production media will be prepared and transferred aseptically via a 1L feed bottle to the bioreactor vessel. After a 24-hour media hold, the media will be inoculated with the appropriate volume of cell culture from the shake flask.

Collect:

- 1L bottle of production medium
- 10mL bottle of 10mg/mL gentamicin

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- D-glucose solution (45%)

7.4.2.1. Preparation of media and feed bottle

7.4.2.1.1. Place production medium, glucose, and gentamycin in BSC

7.4.2.1.2. Place the autoclaved feed bottle in the BSC

7.4.2.1.3. Using a 100ml serological pipette transfer 1L of production medium to the sterile feed bottle

7.4.2.1.4. Using a 10ml serological pipette, transfer 10ml of gentamicin to the feed bottle

7.4.2.1.5. Using a 2ml pipette transfer 1.56ml of D-Glucose solution to the feed bottle

7.4.2.2. Preparation of Applikon Bioreactor and Addition of media

7.4.2.2.1. Remove the Applikon bioreactor vessel from the autoclave and prepare the bioreactor for cultivation according to steps 8.3.1 to 8.3.8 of the Applikon ez-Control Bioreactor Controller

7.4.2.3. Addition of media

7.4.2.3.1. Carefully remove the aluminum foil from the male connector on the 1 L addition bottle and connect the male connector to the female connector on the addition port of the bioreactor

7.4.2.3.2. Open the clamp on the tubing connected to the addition port of the Applikon Bioreactor

7.4.2.3.3. On the Applikon touch screen select Menu > Manual Control > Acid Pump On

7.4.2.3.4. 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 feed 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.

7.4.2.3.5. Once all of the media has transferred into the vessel, turn off the acid pump. On the Applikon touch screen select Menu > Manual Control > Acid Pump Off

7.4.2.3.6. Close the white clamp attached to the tubing of the addition port

7.4.2.3.7. Disconnect the male and female connector of the addition bottle from the addition port of the bioreactor.

7.4.2.3.8. On the Applikon touch screen select Menu > Manual Control > Acid Pump On

7.4.2.3.9. Remove the tubing by gently pulling the tube while the pump is running.

7.4.2.3.10. Turn off the acid pump. On the Applikon touch screen select Menu > Manual Control > Acid Pump Off

7.4.2.4. Turn on the CO_2 tank by turning the top knob on the tank to the left. Set the output pressure at 20 psi. on the tank regulator

7.4.2.5. Connect the remaining parts of the bioreactor to the controller, refer to SOP: Applikon EZ -Control Bioreactor Controller Operation

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7.4.2.6. Input the set points and limits listed in the table below per the bioreactor SOP.

Parameter	pH	Temp (°C)	Stirrer (rpm)
Set Point	7.15	37	150
Upper Limit	7.20	38	151
Lower limit	7.10	36	149

7.4.2.7. When all control loops are at a set point begin 24 hours media hold to check for contamination. Turn on the Temperature and Stirrer controller. Refer to step 8.8.9.6 of the Applikon ez-control bioreactor Controller Operation SOP

7.4.2.8. One the temperature reaches 37^{o}C turn on the pH controller

7.4.2.9. DO probe polarization

7.4.2.9.1. The DO probe should be polarized for a minimum of 6 hours. Polarize DO probe by connecting the probe to the controller. After minimum of 6 hours of polarization (contact with the media, can be done after overnight media hold)), set DO parameters as follows:

Parameter	% DO
Set Point	40
Upper Limit	42
Lower Limit	38

7.4.2.10. After overnight media hold check the media for contamination

7.4.2.11. Turn on the air tank using the knob on the top of the tank and set the pressure at 30 psi.

7.4.2.12. Calibrate the DO probe per the Applikon ez-Control Bioreactor Controller Operation SOP (step 8.3.11-8.3.12).

7.4.3. Cultivation

7.4.3.1. After 24 hrs. of media hold, check media for contamination.

7.4.3.2. From the shake flask viable cell concentration data calculate the appropriate volume for 4.5 x 10^8 cells. This will result in an initial cell concentration of 4 x 10^5 cells/ml.

7.4.3.3. In the BSC aseptically transfer the appropriate volume of cell suspension from shake flask to the prepared inoculum bottle.

7.4.3.3.1. Carefully remove the aluminum foil from the male connector on the inoculum bottle and connect the male connector to the female connector on the addition port of the bioreactor

7.4.3.3.2. Open the clamp on the tubing connected to the addition port of the Applikon Bioreactor

7.4.3.3.3. On the Applikon touch screen select Menu > Manual Control > Acid Pump On

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7.4.3.3.4. As the pump turns, feed the tubing around it. Use care to avoid pinching fingers. Bend the middle if the tubing that is attached to the feed 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.

7.4.3.3.5. Once all of the media has transferred into the vessel, turn off the acid pump. On the Applikon touch screen select Menu > Manual Control > Acid Pump Off

7.4.3.3.6. Close the white clamp attached to the tubing of the addition port

7.4.3.3.7. Disconnect the male and female connector of the addition bottle from the addition port of the bioreactor.

7.4.3.3.8. On the Applikon touch screen select Menu > Manual Control > Acid Pump On

7.4.3.3.9. Remove the tubing by gently pulling the tube while the pump is running.

7.4.3.4. Turn off the acid pump. On the Applikon touch screen select Menu > Manual Control > Acid Pump Off

7.4.3.5. Visually monitor foam accumulation in the culture and add antifoam as necessary. (e.g. 1ml antifoam reagent plus 9ml of production media can be added on day 3 of cultivation)

7.4.3.6. 15 minutes after inoculation (Day 0) and at 24 hr. intervals, sample the culture to determine OD, viable cell count, cell viability, glucose concentration, lactate concentration. Refer to step 7.5

7.4.3.7. **Record the data in the production batch record**.

7.5. Bioreactor Sampling/Monitoring

As in previous steps with the shake flask, after inoculation samples are taken of the culture in the bioreactor immediately (Day 0, 15 minutes immediately after inoculation) and at specified time points to monitor cell growth and viability and culture conditions. Samples from each time point are analyzed using tests for: (1) optical density at 650 nm (2) viable cell concentration (3) glucose and lactate concentration. Samples should be taken daily until the cell density of > $4x10^6$ cells/mL (typically day 6) is reached. At this cell concentration, the conditioned media in the bioreactor is harvested.

7.5.1. Sampling Procedure: Day 0-7

7.5.1.1. For each time point- label:

- 2 - Spectrophotometer cuvettes as “blank" and “sample”
- microfuge tube labelled glucose/lactate, day, team initials
- microfuge tube labelled cell count
- 1 microfuge tube labeled 'Blank"
- 2, 10ml sterile syringe
- 15ml conical tube

7.5.1.2. In the BSC, aseptically transfer 1.1 mL of the blank solution to a microfuge tube labeled blank.

7.5.1.3. Log in to Applikon EZ Controller as operator per Applikon Operator SOP

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7.5.1.4. Spray the sampling tube end cap with 70% EtOH
7.5.1.5. Unscrew the end cap on the sample port tubing
7.5.1.6. Connect a 10ml syringe to the female lure lock on the sample port tubing
7.5.1.7. Open the white clamp such that it allows the liquid to flow
7.5.1.8. Draw 7ml of culture into the syringe to prime the line, dispose of culture in a waste beaker to be bleached
7.5.1.9. Draw 5ml of culture into a new syringe for test sample
7.5.1.10. Disconnect the sample port tubing from the syringe
7.5.1.11. With a 10ml syringe connected to a 0.2u filter unit push clean air into the sample port to expel culture in the sample pipe back into the vessel
7.5.1.12. Close the white clamp and replace the end cap
7.5.1.13. Transfer the 5ml culture sample into a 15ml conical tube
7.5.1.14. Cell Concentration and Viability – Luna Fluorescent cell counter
7.5.1.14.1. Invert sample tube 3 times gently then remove 100ul and place in tube labelled cell count.
7.5.1.14.2. Perform the fluorescent cell counting by Luna as per “SOP: Operation of Logos biosystems Luna-FL Fluorescence Cell Counter for Fluorescence Cell Counting.” Record the viable cells/mL and % Viability in the table of the Batch Record
7.5.1.15. OD Measurement at 650nm
7.5.1.15.1. Invert sample tube 3 times gently then remove 1 mL of the sample from the sample tube and place in spectrophotometer cuvette labeled "sample". Invert sample tube before removing the 1ml
7.5.1.15.2. Measure OD at 650 nm as per steps 7.3.1.10. Record the OD in the table of the Batch Record.
7.5.1.16. Glucose and Lactate concentrations
7.5.1.16.1. Transfer l ml of the sample from the 15ml tube to a 1.5 mL microcentrifuge tube labeled sample. Centrifuge the 1.5 mL tube in the microcentrifuge for 5 minutes.
7.5.1.16.2. Remove 980 µl of supernatant from the sample bottle and transfer to a microfuge tube labeled “Glu/Lac [bioreactor], [time point], [initials], [date]".
7.5.1.16.3. Measure Glucose and Lactate concentration using YSI Bioanalyzer by following: “SOP Measurement of Glucose and Lactate conc in Media using YSI 2500 Bioanalyzer “
7.5.1.16.4. Store the sample at 4°C in a microfuge tube storage box labeled with Date, Team Name, Vessel Name

7.6. End of Run – Culture Harvest
When the cell culture reaches the cell density between 4 x 10^6 and 5 x 10^6 cells/mL typically Day 6 of the run), the conditioned media is harvested.
7.6.1. Disconnect the bioreactor from the controller by following the steps 8.6.1, 8.6.2 and 8.7 of the Applikon EZ-Control Bioreactor Controller Operation SOP.

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7.6.2. Transfer the cell suspension to the sterile 250 mL centrifuge bottles using a 100ml pipette. Record the total volume of cell suspension transferred.

7.6.3. Weigh and match the weight of the 2 centrifuge bottles to balance the weight in the centrifuge.

7.6.4. Centrifuge cells in pre-chilled Sorvall centrifuge, fitted with a SLA1500 rotor, at 2500 x g for 10 min at 4°C

7.6.5. Transfer conditioned medium (CM) from centrifuge bottles to sterile storage bottle by pipetting the supernatant being careful not to disturb the pellet. Record the total volume.

7.6.6. 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 before storage: Add appropriate volume of 100X protease inhibitor cocktail to generate a 1X final concentration.

7.7. Prepare Growth Curves

7.7.1. Using excel plot OD, pH, viable cell concentration, glucose, lactate, vs. time (use 2 y-axes). Attach growth curve to Batch Record

7.7.2. Determine growth rate and doubling time of the 100 mL shake flask culture and 1L bioreactor cultures. Attach calculations to Batch Record.

8. History:

Revision Number	Effective date	Preparer	Description of Change
0	10 Jan 2024	Hetal Doshi & Maggie Bryans	Initial release