SOP: Batch Culture of Anti IL-8 Monoclonal Antibody Secreting CHO DP-12 Cells

Approvals:

Preparer: Hetal Doshi and Cianna Cooper Date: 20MAR20
Reviewer: Robin Zuck Date: 23MAR20
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1. **Purpose:**
   1.1. Batch culture of the CHO DP12 cell line for the production of recombinant human Anti IL-8 monoclonal antibodies. Cells will be cultured in 100ml spinner flask and scaled up to 1L in a bioreactor.
2. **Scope:** Applies to the production of recombinant Anti IL-8 monoclonal antibodies from recombinant Chinese Hamster Ovary (CHO) DP12 clone.
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. CHO DP12-ATCC® CRL-12444/12445 cell line construction culture method https://www.atcc.org/products/all/CRL-12445.aspx
   4.2. SOP: Labconco Purifier Class 2 Biological Safety Cabinet Operation, Document No. UP 1
   4.3. SOP: Bellco Spinner Flask(100ml) Cleaning and Autoclaving, Document No. UP 2
   4.4. SOP: Oakton PC 700 Bench Series pH/Conductivity/°C/°F Meter, Document No MET1
   4.5. SOP: Glucose Determination Assay Using Spectrophotometer, Document No. QCB 3
   4.6. SOP: Lactate Determination Assay Using Spectrophotometer, Document No. QCB 4
   4.7. SOP: Trypan Blue Assay, Document No. UP6
   4.8. SOP: Quantification of CHO DP12 Derived Anti IL-8 Monoclonal Antibody by ELISA, Document No. QCB11
   4.9. 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
      6.1.3. Fisher Scientific Isotemp Low speed magnetic stirrer
      6.1.4. Clean and autoclaved 100 ml Bellco spinner flasks
      6.1.5. Thermo Scientific Biomate UV-visible recording spectrophotometer
      6.1.6. Thermo Scientific Evolution 220 UV-vis spectrophotometer
      6.1.7. Oakton PC 700 Bench Series pH/Conductivity/°C/°F Meter

SOP: Batch Culture of Anti IL-8 Monoclonal Antibody Secreting CHO DP-12 Cells

6.1.8. Fisher Scientific Isotemp 37°C water bath
6.1.9. Fisherbrand microcentrifuge
6.1.10. Nikon E100-LED Compound Light Microscope with 100X magnification (10X objective lens)
6.1.11. Hemocytometer with cover glass
6.1.12. Biorad iMark Microplate reader
6.1.13. Applikon EZ-control bioreactor controller with A 3-liter glass autoclave bioreactor and the processor
6.1.14. 500 ml and 1L liquid addition/feed bottles
6.1.15. 250 ml glass feed bottle for 150 ml alkaline solution
6.1.16. 100 ml glass bottle
6.1.17. Male and female autoclave connectors
6.1.18. Tubing clamps
6.1.19. Gas filters, 0.2µm
6.1.20. Autoclavable silicone tubing size 14(1.6mm interior diameter)
6.1.21. Autoclavable silicone tubing size 16(3.1mm interior diameter)
6.1.22. Autoclavable silicone tubing size 25(4.8mm interior diameter)
6.1.23. Laboratory gasses: Air compressor, CO_2, O_2(optional)

6.2. Materials:
6.2.1. Vials of CHO DP12 cells (ATCC CRL-12445/12444)
6.2.2. Dublecco's Modified Eagle's Medium (DMEM) Corning # 10-013 CV
6.2.3. Superlow IgG Fetal Bovine Serum (Hyclone # SH3089802)
6.2.4. Insulin-Transferrin Selenium (ITS-G) 100X (Gibco # 41400-045)
6.2.5. 0.2mM methotrexate stock solution (1000X) in PBS
6.2.6. Nalagene 250 ml 0.2 µm filter units
6.2.7. Trypan Blue (0.4% solution)
6.2.8. 10X PBS
6.2.9. 150 ml of 1M $NaHCO_3$ (sodium bicarbonate)
6.2.10. 10mg/ml gentamycin
6.2.11. O_2 Electrolyte solution for DO probe
6.2.12. Control serum for glucose and lactate assay
6.2.13. Glucose oxidase assay kit
6.2.14. Lactate assay kit
6.2.15. Glucose standard
6.2.16. Lactate standard
6.2.17. 100ml and 250 ml graduated cylinder
6.2.18. Sterile serological pipettes (2ml, 5ml, 25 ml, and 50 ml)
6.2.19. Pipette aid
6.2.20. Spectrophotometer UV/Vis cuvettes and cuvette rack
6.2.21. Oakton pH 4.0 and pH 7.0 standard buffers
6.2.22. 50 ml beakers
6.2.23. 1-T25 vented tissue culture flask for blank
6.2.24. Test tube rack

SOP: Batch Culture of Anti IL-8 Monoclonal Antibody Secreting CHO DP-12 Cells

6.2.25. 1.5 ml microfuge tube and tube holder
6.2.26. P20, P200, and P1000 micropipettes and compatible tips
6.2.27. Sterile 250ml glass bottles for storage of CHO cell media
6.2.28. Aluminum foil
6.2.29. Autoclave tape

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 CHO DP-12 Complete Growth media- DMEM, 90% Super low IgG Fetal Bovine Serum, 10% 1X Insulin-Transferrin Selenium (ITS-G), 200nM methotrexate solution.

7.1.1. Prepare biological safety cabinet per Labconco Purifier Class 2 Biological Safety cabinet (BSC) Operation SOP

7.1.2. Gather the following items, spray with 70% isopropanol, and place in the biological safety cabinet.

7.1.2.1. Pipette aid (wipe with paper dampened with 70% IPA)
7.1.2.2. 5ml sterile pipettes
7.1.2.3. 25ml sterile pipettes
7.1.2.4. 500ml bottle of pre-sterilized DMEM media
7.1.2.5. Super low IgG Fetal Bovine Serum
7.1.2.6.250 ml sterile 0.22 μm filter unit

7.1.3. 120 ml Complete Growth media:

7.1.3.1. Add 108 ml of DMEM, 12 ml of Super Low IgG FBS, 1.2 ml ITS-G (100X), and 0.120 ml methotrexate (1000X) to the top portion of the filter unit and sterile filter

7.2. Preparation of Spinner flask and Blank tube

7.2.1. Obtain 100 ml Bellco spinner flask that has been previously cleaned and autoclaved per SOP, Bellco Spinner Flask (100ml) Cleaning and Autoclaving

7.2.2. Aseptically transfer 98ml of the prepared complete growth media to the 100ml spinner flask.

7.2.3. Aseptically transfer 20 ml of the prepared complete growth media to a T25 vented tissue culture flask.

7.2.4. Label the spinner flask as CHO DP12, [date], [group#], [Operator initials]. Label the T25 tissue culture flask as BLANK, [date]. [group#], [Operator initials].

7.2.5. Place spinner flask containing CHO cell media in the CO_2 incubator. Set the speed of the magnetic stirrer to 60 rpm to ensure an even mixing of the culture without foaming. Make sure to loosen side arm caps of spinner flask once in incubator.

7.2.6. Place T25 tissue culture flask containing complete growth media in the CO_2 incubator.

7.2.7. Verify that the temperature is 37 ± 0.5°C and percentage of CO_2 is 5 ±0.5%.

7.2.8. Check media for contamination after a minimum of 24 hours.

7.3. Inoculation of Spinner flask

7.3.1. Prepare biological safety cabinet per SOP.

7.3.2. Wipe the pipette with tissue paper dampened with 70% isopropanol and place in the BSC.

SOP: Batch Culture of Anti IL-8 Monoclonal Antibody Secreting CHO DP-12 Cells

7.3.3. Remove spinner flask from the incubator after tightening the side arm caps. Spray spinner flask and place it in the prepared biological safety cabinet for inoculation.

7.3.4. Remove two vials of CHO DP12 cells from storage in the -80°C freezer and record removal of the two vials in the ScienTemp -80°C freezer log. **Each vial should contain between 5 x 10^6 and 10 x 10^6 cells/vial to obtain concentration of 1.8 x 10^5-2.2 x 10^5cells/ml after inoculation.**

7.3.5. Thaw vial contents rapidly by agitation in a 37°C ±0.5°C water bath. **Hold the cryovial in the water without submerging the cap area to avoid contamination.**

7.3.6. Spray vials with 70% isopropanol, and place in the biological safety cabinet.

7.3.7. Aseptically transfer the entire contents of both 1 ml vials of thawed CHO DP12 cells into the Bellco spinner flask labelled CHO DP12 [date], [group#], [operator initials] using 2 ml sterile pipette. Do not add cells to the T25 tissue culture flask labelled BLANK, [date], [group#], [operator initials].

7.3.8. Swirl to mix. Place the spinner flask on the low speed magnetic stirrer set at 60 rpm in the CO_2 incubator at 37°C with 5% CO_2 for 10 minutes. Make sure to loosen side arm caps of spinner flask once in incubator.

7.3.9. Take day 0 samples following the procedure described in 7.4.1.

7.4. Monitoring/Sampling the cell culture

After inoculation, take 3 ml samples of the culture immediately (Day 0, 10 minutes immediately after inoculation) and 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, (pH), (3) viable cell count (trypan blue assay), (4) glucose/lactate concentration and (5) anti IL-8 concentration. Samples will be tested every 24 hrs. + 2 hrs. until the cell density of ≥ 1x 10^6cells/ml (typically day 6) is reached and the culture can then be inoculated into the bioreactor.

7.4.1. Sampling the culture

7.4.1.1.Prepare biological safety cabinet per SOP

7.4.1.2.Collect the following items:

- 5-microfuge tubes labeled “blank”, “cell count”, “trypan blue”, “microcentrifuge counterbalance” and “anti IL-8-vessel name, day of culture, group initials, date”
- 1-microfuge tube holder
- 2-spectrophotometers cuvettes (1 labeled “Sample” and 1 labeled “Blank”)
- 1-cuvette holder
- 1-P1000 pipette
- 1-P20 pipette
- 1 mL pipette aid

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

- 1-15 mL conical tube and conical tube holder
- 1-Microfuge tube labelled blank and microfuge tube holder
- 1-Pipette aid

SOP: Batch Culture of Anti IL-8 Monoclonal Antibody Secreting CHO DP-12 Cells

- 1-5 mL individually wrapped serological pipette
- 1-1 mL individually wrapped serological pipette

7.4.1.4. Remove spinner flask, BLANK T25 tissue culture flask from CO2 incubator, spray 70% IPA and place in biological safety cabinet

7.4.1.5. Using aseptic technique, remove 3 mL of sample from CHO DP12 labeled Spinner Flask and place into the 15 mL conical tube

7.4.1.6. Using aseptic technique, remove 1 mL from BLANK, [date], [group#], [operator initials] and place into a 1.5 mL microfuge tube labeled blank

7.4.1.7. Return CHO DP-12 labeled Spinner Flask and BLANK T25 tissue culture flask to the CO2 incubator, making sure to loosen side arm caps of spinner flask once in incubator

7.4.2. Testing Culture Samples

Collect and analyze 3 mL sample from each time point and test each sample for: (1) optical density at 650 m, (2) viable cell count (trypan blue assay), (3) pH (4) glucose and lactate concentration and (5) anti-IL-8-concentration.

7.4.2.1. Mix 3 mL cell suspension by inverting the 15 mL tube several times. Transfer 100 µl of cell suspension to the tube labelled “cell count”

7.4.2.2. Cell concentration and viability

7.4.2.2.1. Using the 100 µl of cell suspension from microfuge tube labelled “cell count” from the step above determine cell count and cell viability using Trypan Blue Assay SOP

7.4.2.2.2. **Record all data in the production batch record**

7.4.2.3. pH measurement

7.4.2.3.1. Calibrate pH Meter per Oakton PC 700 Bench Series pH/Conductivity/°C/°F Meter SOP

7.4.2.3.2. Using the remaining 2.9 mL sample in the 15 mL conical tube measure the pH reading per Oakton PC 700 Bench Series pH/Conductivity/°C/°F Meter SOP

7.4.2.3.3. Record the pH on data sheet

7.4.2.3.4. Rinse the pH probe with milliQ water and blot dry with kimwipes

7.4.2.3.5. Rinse the pH probe with 70% ethanol and blot dry with kimwipes

7.4.2.3.6. Rinse the pH probe with milliQ water and blot dry with kimwipes. Store the pH probe in the pH storage solution as per pH meter SOP

7.4.2.4. OD Measurement at 650 nm

7.4.2.4.1. Turn on the Biomate 5 UV-Vis spectrophotometer at least 10 minutes prior to measuring the absorbance

7.4.2.4.2. Select general test by pressing the key at bottom of the display screen reading “general test”

7.4.2.4.3. Under the general test menu select “Fixed” by moving the cursor using up/down arrow key. Press “Enter” to select fixed method

7.4.2.4.4. Select wavelength in the fixed method page by using up/down arrow key.

7.4.2.4.5. Using the numeric key enter 650 nm in the popup box. Press ENTER when finished.

7.4.2.4.6. Using the up/down arrow key select “NUMBERS OF SAMPLES” press ENTER.

SOP: Batch Culture of Anti IL-8 Monoclonal Antibody Secreting CHO DP-12 Cells

7.4.2.4.7. Using the numeric key enter 1 in the popup box. Then press ENTER.
7.4.2.4.8. Transfer the 1 mL Blank from microfuge tube into the cuvette labelled "Blank."
7.4.2.4.9. Using the same 2.9 mL of sample from step 7.4.2.1., transfer 1 mL of sample to the cuvette labelled "Sample." Pipet the sample up and down in the cuvette several times to mix.
7.4.2.4.10. Press RUN on the spectrophotometer. Follow the instructions displayed on the screen of the spectrophotometer. Record the reading in the data table of the production batch record.
7.4.2.4.11. Print the result by pressing the button below the print option displayed on the screen
7.4.2.4.12. Remove the sample and blank cuvettes from the spectrophotometer and discard the sample and blank after bleaching the sample with 10 % bleach in the sink.
7.4.2.4.13. Discard the cuvette in the biohazard waste.

7.4.2.5. Anti-IL-8-concentration, Glucose and Lactate Measurement

7.4.2.5.1. Remove 1 mL of the remaining 1.9 mL sample and place in the 1.5 mL micro centrifuge tube labelled "cell". Centrifuge in the microcentrifuge for 5 minutes. Make sure to counterbalance the sample microfuge tube with the microcentrifuge the labeled "counterbalance" containing 1 mL of water.
7.4.2.5.2. Remove the supernatant from the sample and place in the microfuge tube labeled "anti IL-8-vessel name, day of culture, group initials, date". Store at 2-8°C in a microfuge tube storage box labeled with Date, Group Name, Vessel Name for measurement of Anti IL-8, Glucose and Lactate concentration.
7.4.2.5.3. Add 10% Bleach solution to the remaining cell suspension and discard in the Biohazard waste

7.5. Scale up to 1L bioreactor

Note: When the suspension culture of CHO cells reaches a concentration equal to or greater than l x 10^6 cells/mL (typically around day 6), the entire contents of the spinner flask will be added to the bioreactor containing 1L of CHO DP12 Cell Growth Media.

7.5.1. Prepare and autoclave addition/feed bottles

7.5.1.1. Prepare 250 mL alkaline bottle containing 150 mL of 1M $NaHCO_3$ in deionized water

7.5.1.1.1. Weigh out 12.6 ± 0.1g of $NaHCO_3$ and transfer to a 250 mL beaker.
7.5.1.1.2. Using a 250 mL graduated cylinder, measure 100 mL MilliQ water and add to the 12.6 ± 0.1g of $NaHCO_3$ in the beaker. Add a magnetic stir bar and stir on a magnetic stirrer to dissolve.
7.5.1.1.3. Transfer dissolved 1 M $NaHCO_3$ solution to 250 mL graduated cylinder and bring to 150 mL volume with MilliQ water.
7.5.1.1.4. Label the bottle as 1M $NaHCO_3$, [date], [initials], [group number], storage: room temp, disposal: drain.
7.5.1.1.5. Transfer 150 mL solution to the labeled 250 mL alkaline feed bottle

SOP: Batch Culture of Anti IL-8 Monoclonal Antibody Secreting CHO DP-12 Cells

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

7.5.1.2. Prepare 100 mL 1X PBS from 10X PBS stock solution

7.5.1.2.1. In a 100 mL graduated cylinder measure 10 mL 10X PBS and bring to 100 mL volume with 90 mL MilliQ water

7.5.1.2.2. Add 100 mL of 1X PBS to the Bioreactor vessel. (NOTE: Do not autoclave the bioreactor with the Complete Growth Media containing FBS).

7.5.1.3. 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).

7.5.1.4. 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)

7.5.1.5. Autoclave the Applikon bioreactor containing 100 mL of 1X PBS with 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)

7.5.2. Prepare bioreactor for Cultivation

To prepare the bioreactor for cultivation, 1L of Cell Growth 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 cells from the spinner flask.

7.5.2.1. Preparation of 1L Cell Growth Media

7.5.2.1.1. Gather the following items, spray with 70% isopropanol, and place in the biological safety cabinet:

- 1-Pipette aid (swab with tissue papers damped with 70% IPA)
- 1-10 mL sterile pipette
- 2-50 mL sterile pipettes
- 2-500 mL bottle of pre-sterilized DMEM Medium
- 2-50 mL tube of pre-sterilized, Superlow IgG fetal bovine serum (FBS)
- 1 mL aliquot of 1000x methotrexate stock solution
- 10 mL bottle of Insulin-Transferrin Selenium (ITS-G) 100X
- 10mL bottle of 10mg/mL gentamycin
- 2 -Empty 50 mL conical tubes
- 1-autoclaved 1 L addition bottle with tubing and autoclavable male connector attached

7.5.2.1.2. Aseptically remove 100 mL from one 500 mL bottle of pre-sterilized DMEM media and place in the empty two 50 mL tubes.

7.5.2.1.3. Aseptically add the remaining 400 mL and an additional 500 mL bottle of pre-sterilized DMEM media to the 1L addition bottle with tubing and autoclavable male connector attached.

7.5.2.1.4. Aseptically add 100 mL of Superlow IgG FBS to the 1 L addition bottle with tubing and autoclavable male connector attached

SOP: Batch Culture of Anti IL-8 Monoclonal Antibody Secreting CHO DP-12 Cells

7.5.2.1.5. Aseptically add 1 mL of 1000X methotrexate
7.5.2.1.6. Aseptically add 10 mL of ITS-G
7.5.2.1.7. Aseptically add the 10 mL of 10 mg/mL gentamycin.
7.5.2.1.8. Be sure the cap is on tightly and remove the 1 L addition bottle with tubing and autoclavable male connector attached and bring it over to the Applikon bioreactor

7.5.2.2. Preparation of Applikon Bioreactor and Addition of media
7.5.2.2.1. 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

7.5.2.3. Addition of media
7.5.2.3.1. Carefully remove the foil from the female connector on the addition port of the Applikon bioreactor.
7.5.2.3.2. 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
7.5.2.3.3. Remove the clamp on the female connector on the addition port of the Applikon bioreactor
7.5.2.3.4. On the Applikon touch screen select Menu > Manual Control > Acid Pump On
7.5.2.3.5. As the pump turns feed the tubing around it. Use care to avoid pinching fingers.
7.5.2.3.6. 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
7.5.2.3.7. Disconnect the male connector of the addition bottle from the female connector on the addition port of the bioreactor. Bend the tubing of the addition port and reattach the clamp.

7.5.2.4. Turn on the CO2 tank. Set the output pressure at 10 psi. on the tank regulator

7.5.2.5.Connect the remaining parts of the bioreactor to the controller

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

Parameter	pH	Temp (°C)	Stirrer (rpm)
Set Point	7.2	37	75
Upper Limit	7.3	38	76
Lower limit	7.1	36	74

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

7.5.2.8.DO probe polarization
7.5.2.8.1. 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, set DO parameters as follows:

SOP: Batch Culture of Anti IL-8 Monoclonal Antibody Secreting CHO DP-12 Cells

Parameter	% DO
Set Point	50
Upper Limit	52
Lower Limit	48

7.5.2.9. After overnight media hold check the media for contamination
7.5.2.10. Turn on the air pump and set the pressure at 10 psi.
7.5.2.11. Calibrate the DO probe per the Applikon ez-Control Bioreactor Controller Operation SOP (step 8.3.11-8.3.12).

7.5.3. Cultivation

7.5.3.1. After 24 hrs. of media hold, check media for contamination.
7.5.3.2. Inoculate the bioreactor with the cells from the spinner flask by-following the steps in section 8.4.1. of the Applikon ez-Control Bioreactor Controller Operation SOP.
7.5.3.3. Immediately after inoculation (Day 0) and at 24 hr. intervals, sample the culture to determine OD, pH, viable cell count, cell viability. glucose concentration, lactate concentration and concentration of anti-IL-8. (See step 7.6.2 for Day 0-2 and step 7.6.4. for Day 3-End of Run). **Record the data in the production batch record**.

7.6. Bioreactor Sampling/Monitoring

As in previous steps with the spinner flask, after inoculation samples are taken of the culture in the bioreactor immediately (Day 0, 10 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 (trypan blue assay), (3) glucose and lactate concentration and (4) anti IL-8 concentration. Samples should be taken daily until the cell density of > $1x10^6$ cells/mL (typically day 6) is reached. At this cell concentration, the conditioned media in the bioreactor is harvested. **Note: In order to obtain accurate cell concentration and OD readings for Day 0 through Day 2, 25 mL sample will be taken. 5 mL samples will be taken for the later time points (Day 3-End of Run)**

7.6.1. Sampling Procedure: Day 0-2

7.6.1.1.For each time point- label:

- 2 - Spectrophotometer cuvettes as “blank" and “sample”
- microfuge tube labelled “anti-IL-8- bioreactor- time point-initials, date"
- 1 microfuge tube labeled 'Blank"

7.6.1.2.In the BSC, aseptically transfer 1 mL of the blank solution to a microfuge tube labeled blank.
7.6.1.3. Log in to Applikon EZ Controller as operator per Applikon Operator SOP
7.6.1.4. Raise the stirrer upper limit to 150 rpm
7.6.1.5.Change the stirrer setting to 125 rpm.
7.6.1.6. Spray the head plate near the sampling tube with 70% IPA.
7.6.1.7.Remove the black clamp and set on the head plate.
7.6.1.8.Pull out the autoclavable female connector and set it next to the black clamp.

SOP: Batch Culture of Anti IL-8 Monoclonal Antibody Secreting CHO DP-12 Cells

7.6.1.9. Place a 25 mL pipette into the sampling tube, remove 25 mL of sample and place sample in a 50 mL conical tube
7.6.1.10. Put the female autoclavable connector back into the sampling tube.
7.6.1.11. Bend the sampling tubing and place the black clamp back on the tubing.
7.6.1.12. Change the stirrer setting to 75 rpm.
7.6.1.13. Change the stirrer upper limit back to 76 rpm

7.6.2. Testing Culture Samples: Day 0- Day 2

7.6.2.1. OD Measurement at 650nm

7.6.2.1.1. Remove 1 mL of the sample from the 50 ml conical tube and place in spectrophotometer cuvette labeled "sample"
7.6.2.1.2. Aseptically transfer I mL of the blank solution in the BSC to the 1.5 ml Eppendorf tube labelled "Blank" and place in the spectrophotometer cuvette labelled "Blank"
7.6.2.1.3. Measure OD at 650 nm as per step through described in this SOP. Return 1 mL sample back to the 50 mL conical tube. Record the OD in the table of the Batch Record.

7.6.2.2. Cell Concentration and Viability —Trypan Blue Assay

7.6.2.2.1. Centrifuge the 50 mL tube containing sample at 900 rpm for 5 minutes.
7.6.2.2.2. Carefully remove supernatant, leaving approximately 0.2 mL behind so as not to disturb the pellet. Transfer supernatant to a new 50 mL conical tube
7.6.2.2.3. Re-suspend the pellet in 0.8 mL of excess supernatant from step 7.6.2.2.2 using a 5 mL pipette
7.6.2.2.4. Perform the trypan blue assay per SOP on the re-suspended pellet. Record the viable cells/mL and % Viability in the table of the Batch Record

7.6.2.3. Anti- IL-8, Glucose and Lactate concentrations

7.6.2.3.1. Remove 1 mL of supernatant from 50 mL conical tube in step 7.6.2.2.2 and transfer to a microfuge tube labeled “anti IL-8, [bioreactor], [time point], [initials], [date]". Store at 2-8°C in a microfuge tube storage box labeled with Date, Group Name, Vessel Name for measurement of Anti-IL-8, Glucose and Lactate concentrations.

7.6.3. Sampling Procedure: Day 3-End of Run (EOR)

7.6.3.1. Aseptically transfer 1 mL of the Blank solution to the microfuge tube labelled “Blank"
7.6.3.2. Log in to Applikon ez Controller as operator per Applikon Operator SOP
7.6.3.3. Raise the stirrer upper limit to 150 rpm.
7.6.3.4. Change the stirrer setting to 125 rpm.
7.6.3.5. Spray the head plate near the sampling tube with 70% IPA.
7.6.3.6. Remove the black clamp and set on the head plate.
7.6.3.7. Pull out the autoclavable female connector and set it next to the black clamp.
7.6.3.8. Place a 10 mL pipette into the sampling tube and remove 5 mL of sample and place in a 15 mL conical tube.
7.6.3.9. Put the female autoclavable connector back into the sampling tube.
7.6.3.10. Bend the sampling tubing and place the black clamp

SOP: Batch Culture of Anti IL-8 Monoclonal Antibody Secreting CHO DP-12 Cells

7.6.3.11. Change the stirrer setting to 75 rpm
7.6.3.12. Change the stirrer upper limit back to 76 rpm
7.6.4. Testing Culture Samples Day 3- End of Run (EOR)
7.6.4.1.OD measurement at 650nm
7.6.4.1.1. Remove 1 mL of sample from the 15 mL conical tube containing 5 mL sample and place in spectrophotometer cuvette labeled "sample"
7.6.4.1.2. Transfer 1 mL of the blank solution to the spectrophotometer cuvette labelled "Blank"
7.6.4.1.3. Measure OD at 650 nm as per the steps in 7.4.2.4.1. through 7.4.2.4.12. of this SOP and record the data in the table of the production batch record
7.6.4.2.Cell Concentration and Viability -Trypan Blue Assay
7.6.4.2.1. Remove 0.1 mL of the sample from 15 mL conical tube containing the 5 mL sample and perform the trypan blue assay per SOP
7.6.4.3.Anti IL-8, Glucose, Lactate concentration
7.6.4.3.1. Transfer l ml of the sample from the 15 mL conical tube to a 1.5 mL microcentrifuge tube labeled sample. Centrifuge the 1.5 mL tube in the microcentrifuge for 5 minutes.
7.6.4.3.2. Remove 1 mL of supernatant and transfer to microfuge tube labeled "anti IL-8- bioreactor time point- initials, date." Store at 2-8°C in a microfuge tube storage box labeled with Date, Group Name, Vessel Name for measurement of Anti IL-8, Glucose, and Lactate concentrations.

7.7. End of Run – Culture Harvest

When the cell culture reaches the cell density between 0.8 x 10^6 and 1 x 10^6 cells/mL (typically Day 6 of the run), the conditioned media is harvested.

7.7.1. Disconnect the bioreactor from the controller by following the steps 8.6.1, 8.6.2 and 8.7.1 of the Applikon EZ-Control Bioreactor Controller Operation SOP.
7.7.2. Transfer the cell suspension to the 250 mL centrifuge bottles
7.7.3. Centrifuge cells in pre-chilled Sorvall centrifuge, fitted with a SLA1500 rotor, at 500 x g for 5 min, 4°C
7.7.4. Transfer conditioned medium (CM) from centrifuge bottles to storage bottle by pipetting the supernatant being careful not to disturb the pellet.
7.7.5. Sterile filter the conditioned media using the 0.2µm filter unit. Store the filtered condition media in the appropriately labeled storage bottle after addition of protease inhibitors at 4°C for short term and at -20°C for long term. For the detail information regarding the preparation and amount of protease inhibitors refer “SOP: End of Run Anti IL-8 mAb Process: Harvest, Centrifugation, TFF concentration”
7.7.6. Clean bioreactor following steps 8.7.2, 8.7.3, 8.7.4 and 8.7.5 of the Applikon ez-Control Bioreactor Controller Operation SOP

7.8. Determine Glucose, Lactate, and anti IL-8 concentrations

7.8.1. Determine the glucose concentration of all the samples collected from the spinner flask and bioreactor per the Glucose Determination Assay Using Spectrophotometer SOP.
7.8.2. Determine the lactate concentration of all the samples collected from the spinner flask and Bioreactor per the Lactate Determination Assay Using Spectrophotometer SOP

7.8.3. Determine the anti IL-8 mAb concentration of all the samples collected from the spinner flask and Bioreactor using the "SOP: Quantitation of CHO DP-12 derived Anti IL-8 Monoclonal Antibody by ELISA".

7.9. Prepare Growth Curves

7.9.1. Plot OD, pH, viable cells, glucose, lactate, and anti-IL8 concentration vs. time (use 2 y-axes). Attach growth curve to Batch Record

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

8. History:

Revision Number	Effective date	Preparer	Description of Change
0	20 March 17	Cianna Cooper and Hetal Doshi	Initial release
1	24 March 20	Hetal Doshi	Added steps for measuring OD at 650 nm