SOP: CHO DP12 Spinner Flask Culture for the Production of Anti IL-8 Monoclonal Antibody

Approvals:

Preparers: Hetal Doshi — Date: 27JULY21
Reviewer: Robin Zuck — Date: 29JULY21
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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.
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
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
      6.1.8. Fisher Scientific Isotemp 37°C water bath
      6.1.9. Fisherbrand microcentrifuge

6.1.10. Nikon E200-LED Compound Light Microscope with 100X magnification (10X objective lens)
6.1.11. Hemocytometer with cover glass

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. Glucose oxidase assay kit
6.2.9. Lactate assay kit
6.2.10. Glucose standard
6.2.11. Lactate standard
6.2.12. 100ml and 250 ml graduated cylinder
6.2.13. Sterile serological pipettes (2ml, 5ml, 25 ml, and 50 ml)
6.2.14. Pipette aid
6.2.15. Spectrophotometer UV/Vis cuvettes and cuvette rack
6.2.16. Oakton pH 4.0 and pH 7.0 standard buffers
6.2.17. 50 ml beakers
6.2.18. 1-T25 vented tissue culture flask for blank
6.2.19. Test tube rack
6.2.20. 1.5 ml microfuge tube and tube holder
6.2.21. P20, P200, and P1000 micropipettes and compatible tips

7. Procedure:

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% ethanol, 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. 100ml or 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.
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% Ethanol and place in the BSC.
7.3.3. Remove spinner flask from the incubator. Spray spinner flask taking care not to spray the vented caps 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 9 x 10^7 and 1.1 x 10^7 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% ethanol, 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.
7.3.9. Take day 0 samples following the procedure described in 7.4.1.
7.3.10. NOTE: Alternatively, the frozen cells can be used to inoculate T75 flasks, each flask should be inoculated with 2.5 x 10^6 cells. CHO DP12 cells will grow as adherent cells in these flasks. Grow the cells till the flask are about 90% confluent. These cells can be used to later inoculate a spinner flask or the T75 conditioned media can be directly used for downstream processing.

7.4. Monitoring/Sampling the spinner flask 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), and (4) glucose/lactate concentration. Samples will be tested every 24 hrs. + 2 hrs until plateau phase is reached, usually day 5 or day 6 and a cell concentration of 0.8 to 1.2 x10^6 cells/ml before harvesting.

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
- 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.

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.

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.

7.4.2.4.11. Place the cuvette labelled "Blank" in the cell one and cuvette labelled "Sample" in cell two

7.4.2.4.12. Follow the instructions displayed on the screen of the spectrophotometer.

7.4.2.4.13. Record the reading in the data table of the production batch record.

7.4.2.4.14. Print the result by pressing the button below the print option displayed on the screen

7.4.2.4.15. 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.

7.4.2.4.16. Discard the cuvette in the biohazard waste.

7.4.2.5.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 “day #, S, team name and date”. 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 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 Harvest the spinner flask when the cells reaches the concentration of 0.8 to 1.2 x10^6 cells/ml: refer to SOP **Spinner Flask Harvest**

7.6 Prepare Growth Curves

7.6.1 Plot pH, viable cell conc., % viability, glucose, lactate, and vs. time (use 2 y-axes). Attach growth curve to Batch Record

7.6.2. Determine growth rate and doubling time of the 100 mL spinner flask.

7 History:

Revision Number	Effective date	Preparer	Description of Change
0	24May2021	Hetal Doshi	Initial release
1	01Aug2021	Hetal Doshi	Updated cell concentration