SOP: NISTCHO Shake Flask 30ml Batch Culture for Monoclonal Antibody Production

Approvals:

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Reviewer: Kayla Clement Date: 04OCT23

1. **Purpose:**
   1.1. Small-scale batch culture of the NISTCHO cell line for recombinant human anti-RSV monoclonal antibody production.
2. **Scope:** Applies to 30ml culture for production of recombinant anti RSV monoclonal antibody from recombinant Chinese Hamster Ovary NISTCHO cells
3. **Responsibilities:**
   3.1. The course instructor/lab assistant is responsible for ensuring that this SOP is performed as described and for updating the procedure when needed.
   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
   4.2. SOP: Labconco Purifier Class 2 Biological Safety Cabinet Operation, Document No. UP1
   4.3. SOP: Operation of Logos biosystems Luna-FL Fluorescence Cell Counter for Fluorescence Cell Counting, Document No. UP22
   4.4. SOP: Trypan Blue Assay for Cell Viability Determination, Document number UP6 Revision number:2
5. **Precautions:**
   5.1. Use BSL1 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 (BSC)
      6.1.2. CO_2 incubator with shaking capability set at 37°C, 5% CO_2, and 125rpm (e.g., Benchmark incu-shaker™ CO2 mini, with non-slip rubber mat platform, catalog#: h3501)
      6.1.3. 125ml Erlenmeyer PETG flat base shake flask with vent cap, sterile, catalog# CGN-2092 125
      6.1.4. Shake flask holder.
      6.1.5. Luna-FL automated fluorescence cell counter
      6.1.6. Luna reusable slide and coverslip or disposable slide
      6.1.7. 0.2-micron PES filter.
      6.1.8. Benchtop centrifuge prechilled at 4 °C (Eppendorf 5464R)

6.2. Materials:

6.2.1. NISTCHO cell culture at a cell concentration between 2 and 4 x 10^6 cells/ml and viability ≥ 94 %

6.2.2. EX-CELL® Advanced CHO Fed-Batch Medium, Sigma Aldrich, catalog number: 14366C-1000ML

6.2.3. AO/PI Cell Viability Kit, Logos Biosystem, catalog number: F23001

6.2.4. Sterile serological pipettes (2ml, 5ml, 25 ml, and 50 ml)

6.2.5. Pipette aid

6.2.6. Test tube rack

6.2.7. 1.5 ml microfuge tube and tube holder

6.2.8. P20, P200, and P1000 micropipettes and compatible tips

6.2.9. 50 ml conical tubes

6.2.10. Halt™ Protease Inhibitor Cocktail, EDTA-free (100X), Thermo Scientific, Catalog number: 78425

7. Procedure:

7.1. The batch culture shake flask will be inoculated with cells from an existing NISTCHO suspension culture in the exponential phase of growth. Sampling the existing NISTCHO culture in shake flask for measuring cell concentration and viability:

7.1.1. Prepare biological safety cabinet per SOP.

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

- Pipette aid (sanitize with cleaning wipes or 70% IPA)
- 1 ml sterile serological pipette
- Cryovial rack
- 1.5ml microcentrifuge tube

7.1.3. Remove the NISTCHO cells shake flask from the shaking CO_2 incubator.

7.1.4. Place the NISTCHO cells shake flask in the BSC after swabbing with 70% IPA.

7.1.5. Aseptically remove 200µl of the cell suspension with a 1ml pipette after gently swirling the flask to collect a representative sample.

7.1.6. Place the 200ul cell suspension in 1.5ml microcentrifuge tube.

7.1.7. Place the flask back into the shaking incubator.

7.1.8. Determine the cell concentration and cell viability by Luna fluorescent counter (or by trypan blue assay following the appropriate SOP)

7.1.9. Calculate volume of cell suspension required for total of 9 X 10^6 cells. This will allow seeding of the production culture flask at a density of 3 x 10^5 cells/ml.

7.2. Preparation of production culture shake flask.

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

- Sterile Erlenmeyer shake flask.
- EX-CELL Advanced CHO Fed-batch Medium
- Pipette aid
- 25ml serological pipette

7.2.2. Calculate the volume of medium required for the production batch culture by subtracting the volume of cell suspension calculated in step 7.1.9. from 30ml and transfer that volume of medium to a sterile shake flask.

7.2.3. Place the flask in the shaking incubator to pre warm the medium for 15 minutes.

7.3. Inoculation of the flask.

7.3.1. In the biological safety cabinet aseptically transfer the volume of cell suspension calculated in step 7.1.9. from NISTCHO cell culture to the flask prepared in step 7.2.2. containing pre warmed fed batch medium. Labelled the flask as NISTCHO batch production, passage number, date, time of inoculation and initials.

7.3.2. Incubate the flask in the shaking incubator for 30 minutes.

7.3.3. After 30 minutes sample the NISTCHO production flask by following the steps described in step 7.1.

7.3.4. Record the cell concentration and cell viability.

7.3.5. Monitor the cell growth daily by measuring the cell concentration and cell viability every 24 hrs. ± 2hrs. as described in step 7.1. and create a growth curve.

7.3.6. Harvest culture after it reaches early decline phase when cell viability is ≤ 75%

7.4. Harvest

7.4.1. Prepare the biological safety cabinet per SOP.

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

- 50ml conical tubes and tube holders
- NISTCHO shake flask culture.
- 25ml, 5ml and 1ml sterile serological pipettes
- Halt protease inhibitor cocktail
- 0.2 µm PES membrane vacuum filter unit

7.4.3. Aseptically transfer the entire cell suspension from NISTCHO production flask to a 50 ml conical tube. Record the volume of the culture transferred.

7.4.4. Centrifuge the 50 ml conical tube with the cell suspension at 2440 X g for 5 minutes at 4^{o} C in pre chilled Eppendorf 5464R centrifuge. Balance with a balance tube.

7.4.5. Take the 50ml conical tube out of the centrifuge swab it with 70% ethanol and place it in the BSC.

7.4.6. Transfer the clarified medium to the new 50ml conical tube without disturbing the cell pellet. Record the volume of the medium transferred.

7.4.7. Filter the clarified media using a 0.2 µm PES membrane sterile filter unit.

7.4.8. Add the appropriate volume of Halt protease inhibitor cocktail (100X) to a get final concentration of 1X.

7.4.9. Label the sterile filtered clarified medium container as NISTCHO clarified media, with passage number, harvest date, volume in ml, and initials.

7.4.10. Store at 2-8°C for up to 7 days

7.4.11. Do NOT freeze the clarified medium.

7.4.12. Proceed to downstream processing.

7.5 Prepare Growth Curves

7.5.1. Plot viable cell conc., and % viability, vs. days in culture (use 2 y-axes).

8 History:

Revision Number	Effective date	Preparer	Description of Change
0	04OCT23	Hetal Doshi	Initial release