SOP: NISTCHO Culture for Monoclonal Antibody Production in a T75 Flask

Approvals:

Preparer: Shanna Milligan Date: 03OCT23
Reviewer: Hetal Doshi Date: 24JAN24
Reviewer: Dr. Maggie Bryans Date: 25JAN24

1. **Purpose:**
   1.1. T75 flask culture of the NISTCHO cell line to produce recombinant human anti RSV monoclonal antibody (cNIST mAB)
2. **Scope:** Applies to T75 flask 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. The students/technician is responsible for following the SOP as described and for informing 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 cNIST mAb Guidance Document https://tsapps.nist.gov/srmext/certificates/10197.pdf
   4.2. SOP: Labconco Purifier Class 2 Biological Safety Cabinet Operation, Document No. UP 1
   4.3. SOP: Operation of Logos biosystems Luna-FL Fluorescence Cell Counter for Fluorescence Cell Counting, Document No. UP22
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
      6.1.3. T75 flask with vent cap, sterile, tissue culture treated, Fisher brand catalog# FB012937
      6.1.4. EVOS XL Core Inverted Microscope
      6.1.5. Luna-FL automated fluorescence cell counter
      6.1.6. Luna reusable slide and coverslip or disposable slide
   6.2. Materials:
      6.2.1. Actively growing NISTCHO cell culture at a cell density of 2-4 x 10^6 cells/ml and viability $\geq$ 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 (1ml, 2ml, 10ml, and 25 ml)

6.2.5. Pipette aid
6.2.6. 1.5 ml microfuge tube and tube holder quantity 1
6.2.7. 50ml conical tubes quantity 2
6.2.8. Halt TM protease inhibitor cocktail, EDTA free (100X) Thermo Scientific, Catalog number: 78425

7. Procedure:

The T75 flask will be inoculated with cells from an existing NISTCHO suspension culture in the exponential phase of growth.

7.1. Determine the cell concentration of the NISTCHO suspension culture and calculate the volume of culture needed to seed the T75 with 3 $x10^5$ cells

7.2. Preparation of production culture T75 flask.

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

- Pack of Sterile T75 flask
- EX-CELL Advanced CHO Fed-batch Medium
- 50ml conical tube
- Pipette aid
- 25ml serological pipette

7.2.2. Subtract the volume of cell suspension calculated in 7.1. from 15ml to determine the volume of medium to add to the T75 flask and transfer that volume of medium to the T75

7.2.3. Place the flask in the incubator set at 37°C with 5% CO_2 to pre-warm the medium for 15 minutes.

7.3. Inoculation of the flask and cell growth

7.3.1. In the biological safety cabinet aseptically transfer the volume of cell suspension calculated in step 7.1. from the NISTCHO cell culture to the T75 flask prepared in step 7.2. containing pre warmed fed batch medium. Pipette up and down gently without generating the bubbles. Label the flask as NISTCHO batch production, passage number, date, time of inoculation and initials.

7.3.2. Incubate the flask in the incubator set at 37°C with 5% CO_2 for 30 minutes

7.3.4. After 30 minutes remove flask from incubator and view the cells under the microscope.

7.3.5. Monitor the cell growth daily by viewing under the microscope and saving the image every 24h ± 2hrs. Cells will remain rounded, and some will be loosely adhered to the surface of the flask

7.3.6. Harvest the culture 7 days after inoculation.

7.4. Harvest

7.4.1. In the BSC gather the following items, spray or wipe with 70% isopropanol, and place in the biological safety cabinet.

- 2 50ml conical tubes and tube holders
- NISTCHO T75 flask culture
- 25ml, 5ml and 1ml sterile serological pipettes and pipette aid
- Halt protease inhibitor cocktail
- 0.2 µm PES membrane vacuum filter unit

7.4.2 Aseptically transfer the entire cell suspension from NISTCHO T75 flask to a 50ml conical tube.
7.4.3. Centrifuge the 50ml conical tube with the cell suspension at 2440 X g for 5 minutes at 4°C in pre chilled Eppendorf 5464R centrifuge. Balance with a balance tube.
7.4.4. In the BSC carefully transfer the clarified medium to a new 50ml conical tube without disturbing the cell pellet. Record the volume of the medium transferred.
7.4.5. Filter the clarified media using a 0.2 µm PES membrane sterile filter unit using a vacuum.
7.4.6. Add the appropriate volume of Halt protease inhibitor cocktail (100X) to a get final concentration of 1X
7.4.7. Label the sterile filtered clarified medium container as NISTCHO clarified media, with passage number, harvest date, volume in ml, and initials.
7.4.8. Store at 2-8°C for up to 7 days, Do NOT freeze the clarified medium.
7.4.9. Proceed to downstream processing.

8. History:

Revision Number	Effective date	Preparer	Description of Change
0	25JAN25	Shanna Milligan	Initial release