SOP: Resuscitation and Culture of NISTCHO Cells

Approvals:

Preparer: Hetal Doshi Date: 25JAN24
Reviewer: Dr. Maggie Bryans Date: 29JAN24

1. Purpose:

1.1. This Standard Operating Procedure (SOP) describes the steps required for the cultivation of NISTCHO Cells in suspension under BSL-1 safety criteria. NISTCHO cells are Chinese hamster ovary (CHO) cell line that has been engineered by Millipore Sigma to express a recombinant anti RSV cNIST monoclonal antibody (mAb)

2. Scope:

This SOP will be applied to resuscitation and initial cultivation of NISTCHO cells in suspension culture.

3. Summary of Method:

3.1. Pre-warm the media
3.2. Thaw the NISTCHO cell cryovial.
3.3. Wash the cells with pre warmed media to remove DMSO.
3.4. Initiate NISTCHO cell culture

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. UP 1
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, Document No. UP6
4.5. Thawstar CFT 2 thawing system user manual 10000010333-ThawSTAR® CFT2 Automated Thawing System (stemcell.com)

5. Definitions:

5.1. N/A

6. Precautions:

6.1. Use BSL1 safety measures and practices and discard waste in biohazard containers after adding bleach.
6.2. 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.
6.3. Gloves, a lab coat and protective eyewear should be worn when handling buffers and samples.

7. Responsibilities:

7.1. The course instructor/lab assistant is responsible for ensuring that this SOP is performed as described and for updating the procedure when needed.
7.2. 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.

8. Equipment and Materials:

8.1. **Equipment:**

8.1.1. Biological safety cabinet Class II (BSC)
8.1.2. Thawstar CFT2 thawing system or Water bath set at 37 ^{o}C.

SOP: Resuscitation and Culture of NISTCHO Cells

8.1.3. CO_2 incubator with shaking capacity set at 37°C ±2, 5% CO_2 (e.g., Benchmark incu-shaker™ CO2 mini, with non-slip rubber mat platform catalog# sku: H3501)
8.1.4. Eppendorf centrifuge 5804R
8.1.5. EVOS XL Core imaging system (or similar make inverted digital microscope)
8.1.6. Luna-FL Fluorescence Cell Counter
8.1.7. Nikon E200-LED Compound Light Microscope with 100X magnification (10X objective lens) (optional)
8.1.8. Hemocytometer (optional)
8.1.9. Vacuum pump

8.2. **Material:**

8.2.1. NISTCHO Cell Vial(s) (RGTM 10197)
8.2.2. EX-CELL® CD CHO Fusion medium, Sigma Aldrich catalog #14365C-1000ML, (Will be referred as expansion medium)
8.2.3. Sterile 125-mL PETG Erlenmeyer shaker flask with HDPE Vent Cap, Sterile Chemglass Catalog # CGN-2092-125
8.2.4. Pipette aid
8.2.5. Cryovial rack
8.2.6. Sterile serological pipettes (2ml, 5ml and 25 ml)
8.2.7. Lab coat, gloves, sleeves
8.2.8. 70% Isopropanol or Ethanol
8.2.9. Lint free clean room wipes
8.2.10. Kim Wipes
8.2.11. Trypan Blue (0.4% solution)
8.2.12. 1.5 ml microfuge tube and tube holder
8.2.13. 15ml conical tube with stand
8.2.14. 15ml conical tube with 10ml milliQ water to use as a blank
8.2.15. Autoclaved glass Pasteur pipette
8.2.16. 125ml shake flask holder (magnetic)
8.2.17. Acridine Orange/Propidium Iodide Stain Logos Biosystem, Catalog # F23001

9. Procedure:

9.1. Preparation of the shake flask

9.1.1. Prepare the BSC as per the SOP
9.1.2. Turn on the water bath or Thawstar if not done already.
9.1.3. Collect the following material and place it in the biological safety cabinet after swabbing or wiping them with 70% Ethanol.

- Pack of sterile 15ml conical tube
- Conical tube rack
- Cryovial rack
- Sterile serological pipette sizes 2ml, 5ml, 10ml, and 25ml
- Pipette aid
- Expansion Medium (EX-CELL® CD CHO Fusion medium, Sigma Aldrich catalog #14365C-1000ML

SOP: Resuscitation and Culture of NISTCHO Cells

- Sterile 125ml Erlenmeyer shake flask.
- Autoclaved glass Pasteur pipette (if not already in the BSC)

9.1.4. In BSC, loosen the caps of shake flask, medium bottle and 15ml conical tube. Aseptically transfer 30ml of expansion medium to the shake flask.

9.1.5. Aseptically transfer 9ml of expansion medium to the 15ml conical tube.

9.1.6. Place the shake flask and 15ml conical tube with expansion medium to a shaking incubator for 15 minutes to pre warm the medium. Store the medium bottle at 4°C.

9.1.7. After 15 minutes remove the 15 ml conical tube from the incubator and place it in the BSC after swabbing with 70% Ethanol

9.2. Thawing of the cells

9.2.1. Collect the NISTCHO cryovial from -150°C freezer and thaw the cells in the Thawstar CFT2 following the Thawstar protocol or in the water bath set at 37°C. (Note: if using the water bath hold the vial in such a way that cap of the vial is above the water level to avoid contamination)

9.2.2. Note the details of the vial of cells such as passage number, date frozen, and cell concentration.

9.2.3. Transfer the vial of cells to the Biosafety Cabinet after swabbing the outside of the vial with a Kimwipe moistened with 70% alcohol and place in the cryovial rack.

9.2.4. Using sterile 2ml serological pipette aseptically transfer the cells from the cryovial to the 15 ml conical tube. Rinse the cryovial with 1ml of medium from the 15ml conical tube and add the 1ml back to the 15ml conical tube.

9.2.5. Centrifuge 15ml conical tube with cells in the Eppendorf centrifuge at 220 x g for 5 minutes at 15-20°C after balancing the tube with 15ml balance tube.

9.2.6. Meanwhile remove the shake flask with the media from the incubator and place it in the BSC after swabbing with 70% Ethanol.

9.2.7. Remove the 15ml conical tube with cells and place it in the BSC after swabbing with 70% ethanol.

9.2.8. Aseptically remove the supernatant with a sterile glass Pasteur pipette attached to the vacuum pump, being careful not to disturb the cell pellet. If needed leave some supernatant in the tube to avoid disturbing the cell pellet

9.2.9. Using a sterile 5ml serological pipette aseptically transfer 5ml of expansion medium from the shake flask to the 15ml tube and resuspend the cells by gently pipetting up and down to achieve a single cell suspension. (Note: Avoid creating bubbles)

9.2.10. Aseptically transfer the cell suspension from the 15ml tube to the shake flask. Label the flask with NISTCHO, passage number, date, and initials.

9.2.11. Place the shake flask with the cells in the shaking incubator.

9.3. Monitoring/sampling the cells.

9.3.1. 30 minutes after inoculation and every 24h ± 2h post inoculation, aseptically remove 200µl of culture using a 1ml pipette and transfer to a sterile microfuge tube in the BSC.

9.3.2. Perform a cell count and cell viability assay using the Luna-FL Fluorescence Cell Counter following the SOP. Record the viable cell concentration and % viability.

SOP: Resuscitation and Culture of NISTCHO Cells

9.4. Subculture of NISTCHO cells

Once the culture reaches 3-4 x 10^6 cells/ml the cells are sub cultured. (Approximately 98h post seeding)

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

9.4.2. Calculate the volume of cell suspension required to seed a new shake flask at 0.2 to 0.3 x 10^6cells/ml using the cell count obtained from step 9.3.2. Calculate amount of expansion medium required to seed new flask by subtracting the volume of cell suspension from 30ml.

9.4.3. Collect and place the following material after swabbing or wiping with 70% ethanol.

- Bottle of expansion medium
- Sterile individually wrapped serological pipettes (25ml, 5ml, 2ml and 1ml)
- Sterile 125 ml Erlenmeyer shake flask.
- Pipette aid

9.4.4. Aseptically transfer the calculated volume of medium in step 9.4.2. to the new shake flask

9.4.5. Place the new shake flask in the shaking incubator to prewarm the medium.

9.4.6. After 15 minutes place the new shake flask with medium and shake flask with NISTCHO cells in BSC after swabbing with 70% ethanol.

9.4.7. In BSC, the calculated amount of cell suspension from step 9.4.2. from the NISTCHO cell flask to the shake flask containing expansion medium. Label the flask as NISTCHO, passage number, date, and initials.

9.4.8. The cells can be used for expansion of the culture, batch production of cNIST mAb or can be cryopreserved to generate a cell bank.

10. History:

Revision Number	Effective Date	Preparer	Description of Change
0	29JAN24	Hetal Doshi	Initial release