SOP: Cryopreservation of NISTCHO Cells

Approvals:

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Reviewer: Dr. Maggie Bryans — Date: 01NOV2024

1. Purpose:

1.1. This Standard Operating Procedure (SOP) describes the steps required for the cryopreservation of NISTCHO cells. NISTCHO cells are a Chinese hamster ovary (CHO) cell line that has been engineered by MilliporeSigma to express a recombinant anti RSV monoclonal antibody (cNISTmAb)

2. Scope:

2.1. This SOP will be applied to cryopreservation of NISTCHO in suspension when cell culture reaches a density of $3 \times 10^6 - 4 \times 10^6$ viable cells/mL (SOP: Resuscitation and Culture of NISTCHO cells)

3. Summary of Method:

3.1. Determine the cell concentration and viability of the NISTCHO culture in the log phase.

3.2. Calculate and prepare the amount of cryopreservation medium required to cryopreserve the cells at 1.3×10^7cells/ml concentration.

3.3. Centrifuge the appropriate amount of cell suspension and resuspend them in a appropriate volume of cryopreservation medium

3.4. Place the vials in freezing cube/Mr. frosty and store at -80˚C overnight and transfer to the -150˚C freezer or LN2 freezer.

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: Resuscitation and Culture of NISTCHO Cells

5. Definitions:

5.1. N/A

6. Precautions:

6.1. Use BSL1 safety measures and practices and discard waste in biohazard containers

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

7. Responsibilities:

7.1. 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.

7.2. 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.

8. Equipment and Materials:

8.1. **Equipment:**

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8.1.1. Biological safety cabinet Class II (BSC)
8.1.2. CO_2 incubator with shaking capacity set at 37°C ±2, 5% CO_2, and 125rpm (e.g., Benchmark incu-shaker™ CO2 mini, with non-slip rubber mat platform catalog# sku: H3501)
8.1.3. Eppendorf centrifuge 5804R
8.1.4. Luna-FL Fluorescence Cell Counter
8.1.5. Vacuum pump
8.1.6. Ultra-Low temperature freezer
8.1.7. Controlled Freeze Cubes, Fisherbrand™ Catalog # 13-131-014

8.2. **Materials:**
8.2.1. NISTCHO cell culture 3 X 10^6 cells/ml- 4 X 10^6 cells/ml cell concentration with a viability of ≥ 95%
8.2.2. EX-CELL® CD CHO Fusion medium, Sigma Aldrich catalog #14365C-1000ML, (Will be referred as expansion medium)
8.2.3. Dimethyl Sulfoxide, Fisher Bioreagents, Catalog# BP231-100
8.2.4. Cryovials, Corning, Catalog # 8670
8.2.5. Pipette aid.
8.2.6. Cryovial rack
8.2.7. Sterile serological pipettes (2ml, 5ml and 25 ml)
8.2.8. Lab coat, gloves, sleeves
8.2.9. 70% Isopropanol or Ethanol
8.2.10. Lint free clean room wipes
8.2.11. Kim Wipes
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 appropriate volume of milliQ water to use as a blank for centrifuge.
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
8.2.18. Cryovial marker
8.2.19. 0.22-micron PES membrane syringe filter
8.2.20. Sterile syringe

9. Procedure:

9.1. Determination of cell concentration and cell viability of the existing NISTCHO cell culture
9.1.1. Prepare the BSC as per the SOP and verify the controlled freezer cube is at room temperature.
9.1.2. Collect the following material and place it in the biological safety cabinet after swabbing or wiping them with 70% Ethanol.
- Sterile serological pipette size 1ml
- Pipette aid
- 1 1.5 ml sterile microfuge tube

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- Cryovial rack

9.1.3. Remove shake flask with NISTCHO cells from 37°C incubator with 5% CO_2 and place the flask in the BSC after swabbing it with 70% Isopropanol. Aseptically transfer 200 µl of the cell suspension to a sterile microfuge tube for determination of viable cell count and cell viability.

9.1.4. Place the shake flask back into the 37°C incubator with 5% CO_2 shaking at 125 rpm.

9.1.5. Determine the viable cell density and viability using Luna-FL Fluorescence Cell Counter

9.2. Preparation of Cryopreservation Medium

9.2.1. Calculate the volume of cryopreservation medium needed for cryopreservation of cells to a final density of 1.3 X 10^7cells/ml/vial (Volume based on total number of cells available for freezing)

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

- Pack of sterile 15ml conical tube
- Conical tube rack/holders
- 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
- Dimethyl Sulfoxide
- Autoclaved glass Pasteur pipette (if not already in the BSC)
- Sterile syringe
- 0.22-micron Syringe filter
- Pack of sterile 50 ml conical tube
- Conical tube with appropriate amount of milliQ water to be used as a blank for centrifuge

9.2.3. Prepare the appropriate volume of cryopreservation medium with 93% Expansion Medium+ 7% DMSO. (volume calculated in step 9.2.1. plus 2ml)

9.2.4. Sterile filter the prepared cryopreservation medium with 0.2 µm syringe filter or bottle top filter depending on volume

9.2.5. In BSC label appropriate number of vials as “NISTCHO cells, Passage number, cell concentration/ml/vial, Date, and initials”

9.2.6. Remove shake flask with NISTCHO cells from 37°C incubator with 5% CO2 and place the flask in the BSC after swabbing it with 70% Isopropanol.

9.2.7. Aseptically transfer the appropriate volume of cell suspension to the conical tube

9.2.8. Balance tube and centrifuge at 220 x g for 5 minutes at 20°C

9.2.9. Place the tube in BSC after spraying with 70% Isopropanol. In the BSC discard the supernatant without disturbing cell pellet

9.2.10. Resuspend pellet in 10% of final banking volume of cryopreservation medium prepared in step 9.1.5.

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9.2.11. Adjust the volume with cryopreservation medium to obtain the desired cell density of 1.3 X 10^7 cells/ml

9.2.12. Aliquot 1mL of cell suspension into 2mL screw-capped cryovials. Apply the cap to the cryovials, seal well.

9.2.13. Place the vials in the Controlled Freeze Cube

9.3. Freeze the cells at -80°C overnight prior to transferring the frozen cells to -150°C.

10. History:

Revision Number	Effective Date	Preparer	Description of Change
0	01NOV2024	Hetal Doshi	Initial release