SOP: Cryopreservation of Suspension Viral Production Cells 2.0 - HEK293F

Approvals:

Preparer: Isso Bayala & Hetal Doshi Date: 15AUG22
Reviewer: Dr. Maggie Bryans Date: 16AUG22

1. Purpose:

1.1. This Standard Operating Procedure (SOP) describes the steps required for the cryopreservation of Viral Production Cells 2.0 in suspension under BSL-2 safety criteria. Viral production Cells 2.0 Prototype are a clonal derivative of HEK293F cell line and have been adapted to suspension, high-density culture in Gibco Viral Production Medium. These cells can be thawed directly into Gibco Viral Production Medium

2. Scope and Applicability:

2.1. This SOP will be applied to cryopreservation of viral production cells 2.0 cells in suspension when cell culture reaches a density of 4 X 10^6 - 6 X 10^6 viable cells/mL (SOP: Resuscitation and Culture of Viral Production Cells (VPC) 2.0 -HEK293F UP 32)

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. Clonal HEK293F-derived cell line adapted for AAV production user manual https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2FMAN0019620_ViralProductionCells_2-0_and_ViralProductionMedium_UG.pdf

4.2. SOP: Labconco Purifier Class II Biological Safety Cabinet Operation, Document No. UP 1

4.3. SOP: Bio-Rad TC20 Cell Counter

4.4. 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. Liquid Nitrogen Dewar

6.1.3. Equipment to determine cell viability (cell counter or hemocytometer)

6.1.4. centrifuge

6.1.5. Compound Light Microscope with 100X magnification (10X objective lens)

6.1.6. Ultra-Low temperature freezer

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6.2. Materials

6.2.1. Viral Production Cell (VPC) Culture
6.2.2. 2.0 mL screw-capped cryovials.
6.2.3. Gibco viral production Medium A49842DK
6.2.4. Gibco GlutaMAX Supplement (100X)35050061
6.2.5. HPLC grade dimethyl sulfoxide (DMSO)
6.2.6. Sterile 50-mL centrifuge tubes
6.2.7. Pipette aid
6.2.8. Sterile serological pipettes (2ml, 5ml and 25ml)
6.2.9. Lab coat, gloves, sleeves
6.2.10. 70% Isopropanol
6.2.11. Sterile cleaning wipes
6.2.12. Trypan Blue (0.4% solution)
6.2.13. 1.5 ml microfuge tube and tube holder
6.2.14. P20 micropipettes and compatible tips

7. Procedure:

7.1. Preparation of freezing Media

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 or wipe with 70% Isopropanol, and place in the biological safety cabinet.

- Pipette aid (sanitize with cleaning wipes or 70% IPA)
- 25ml sterile pipettes
- 5ml sterile pipettes
- 1 ml sterile pipette
- 125-mL PETG Erlenmeyer shaker flask
- Cryovial rack
- Gibco viral production Medium
- GlutaMax supplement 200mM
- DMSO
- 1.5 ml Eppendorf tube

7.1.3. Prepare the freezing medium with 90% Viral Production Medium supplemented with 4mM GlutaMAX™ Supplement + 10% DMSO.
7.1.4. Sterile filter the prepared media with 0.2 µm syringe filter and keep on ice until ready to use

7.2. Remove VPCs 2.0 shaker flask from 37°C incubator with 8% CO_2 and place the flask in the BSC after swabbing it with 70% Isopropanol. Aseptically transfer remove 200 µl of the cell suspension for determination of viable cell count and cell viability
7.3. Place the shaker flask back into the 37°C incubator with 8% CO2
7.4. Determine the viable cell density and viability using automated cell counter

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7.5. Calculate the volume of freezing media needed for cryopreservation of cells to a final density of 1 X 10^7cells/ml
7.6. Remove VPCs 2.0 shaker flask from 37°C incubator with 8% CO2 and place the flask in the BSC after swabbing with 70% Isopropanol. Aseptically transfer appropriate amount of cell suspension into a sterile 50mL centrifuge tube in the BSC
7.7. Balance tube and centrifuge at 300 × *g* for 5 minutes.
7.8. Place the tube in BSC after spraying with 70% Isopropanol. In the BSC discard the supernatant without disturbing cell pellet.
7.9. Resuspend pellet in 10% of final banking volume of cryopreservation medium prepared in step 7.1.3.
7.10. Adjust the volume with cryopreservation medium to obtain the desired cell density of 1 X 10^7 cells/ml.
7.11. Aliquot 1-mL of cell suspension into 2.0 mL screw-capped cryovials. Apply the cap to the cryovials, seal well
7.12. Place the vials in Mr. Frosty
7.13. Freeze the cells at -80°C for overnight prior to transfer the frozen cells to liquid nitrogen.
7.14. Transfer frozen the vials to liquid nitrogen for long-term storage

8. History:

Revision Number	Effective Date	Preparer	Description of Change
0	13APR22	Isso Bayala, Hetal Doshi	Initial release