SOP: Resuscitation and Culture of Viral Production Cells 2.0 -HEK293F

Approvals:
Preparers: Isso Bayala & Hetal Doshi — Date: 15AUG22
Reviewer: Dr. Maggie Bryans — Date: 17AUG22

1. **Purpose:**
   This Standard Operating Procedure (SOP) describes the steps required for the cultivation of Viral Production Cells 2.0 in suspension under BSL-2 safety criteria. Viral Production Cells 2.0 Prototype are a clonal derivative of the 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:** This SOP will be applied to initial cultivation of viral production cells 2.0 cells in suspension

3. **Summary of Method:** Viral Production Cells 2.0 Prototype are a clonal derivative of the 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.
4. **Responsibilities:**
   4.1. 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.
   4.2. 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.
5. **References:**
   5.1. AAV-MAX Helper-Free AAV Production System Kit Catalog number: A51217 user manual https://www.thermofisher.com/document-connect/document-connect.html?url=https://assets.thermofisher.com/TFS-Assets%2FLSG%2Fmanuals%2FMAN0019619_AAV-MAX_Helper-Free_AAV_ProductionSystem_UG.pdf
   5.2. SOP: Labconco Purifier Class II Biological Safety Cabinet Operation, Document No. UP 1
   5.3. SOP: Bio-Rad TC20 Cell Counter
   5.4. SOP: Trypan Blue Assay, Document No. UP6
   5.5. SOP: Operation of Logos biosystems Luna-FL Fluorescence Cell Counter for Fluorescence Cell Counting Document Number: UP22
6. **Precautions:**
   6.1. Use BL2 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. **Equipment and Materials:**
   7.1. Equipment
      7.1.1. Biological safety cabinet
      7.1.2. CO_2 incubator shaker

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- 7.1.3. Equipment to determine cell viability (automated cell counter or hemocytometer)
- 7.1.4. Fisher Scientific Isotemp 37°C water bath
- 7.1.5. Fisher brand microcentrifuge
- 7.1.6. Nikon E200-LED Compound Light Microscope with 100X magnification (10X objective lens)
- 7.1.7. Hemocytometer cover glass

7.2. Materials:

- 7.2.1. Vials of Viral Production Cells 2.0 Gibco catalog #A49784 (HEK293F cells)
- 7.2.2. Gibco viral production Medium catalog#A4817901
- 7.2.3. GlutaMAX™ Supplement 200mM Gibco Catalog #35050061
- 7.2.4. Sterile 125-mL PETG Erlenmeyer shaker flask with HDPE Vent Cap, Sterile Chemglass Catalog # CGN-2092-125
- 7.2.5. Pipette aid
- 7.2.6. Cryovial rack
- 7.2.7. Sterile serological pipettes (2ml, 5ml and 25 ml)
- 7.2.8. Lab coat, gloves, sleeves
- 7.2.9. 70% Isopropanol
- 7.2.10. Sterile cleaning wipes
- 7.2.11. Trypan Blue (0.4% solution)
- 7.2.12. 1.5 ml microfuge tube and tube holder
- 7.2.13. P20 micropipettes and compatible tips

8. Procedure:

8.1. Inoculation of the Shaker Flask

- 8.1.1. Prior to use, the medium requires supplementation with 4 mM GlutaMAX Supplement
  - 8.1.1.1. Prepare biological safety cabinet per Labconco Purifier Class 2 Biological Safety cabinet (BSC) Operation SOP
  - 8.1.1.2. 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
    - 125-mL PETG Erlenmeyer shaker flask
    - Cryovial rack
    - Gibco viral production Medium
    - GlutaMax supplement 200mM
  - 8.1.1.3. In BSC aseptically transfer 29.4 ml of Gibco viral production medium to 125 ml PETG Erlenmeyer shaker flask.
  - 8.1.1.4. Aseptically transfer 0.6 ml of 200mM GlutaMax supplement to the Erlenmeyer shaker flask containing Gibco viral production medium

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8.1.1. Label the shaker flask as VPC 2.0, [date], [group#], [Operator initials].

8.1.2. Remove a vial of VPCs 2.0 from liquid nitrogen and swirl in a 37°C water bath for 1 to 2 minutes to thaw the cells rapidly until only a small amount of ice remains N**ote: Do not submerge the vial in the water**

8.1.3. Spray the vial with 70% Isopropanol and place it in the BSC.

8.1.4. Use 5-mL pipette, to aseptically transfer the entire contents of the cryovial into the shaker flask prepared in step 8.1.1.4.

8.1.5. Place the shaker flask containing complete growth media and PVC 2.0 in the CO_2 incubator shaker

8.1.6. Verify that the temperature is 37 ± 0.5°C and percentage of CO_2 is 8 ±0.5%

8.1.7. Set the shake speed to 125±5 rpm.

8.2. Monitoring/Sampling the shaker flask cell culture

After 3 to 4 days post inoculation, take 100 µL samples to monitor cell growth and viability until Cell viability is ≥90% with a viable cell density >1 × 10^6 viable cells/mL

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

8.2.2. Collect the following items, spray with 70% IPA and place in Biological Safety Cabinet:

- 1-microfuge tube
- microfuge tube holder
- 1-P20 pipette
- pipette aid
- 1 mL individually wrapped serological pipette

8.2.3. Remove shaker flask, from CO_2 incubator, spray 70% IPA and place in biological safety cabinet

8.2.4. Using aseptic technique, remove100 µL of sample from shaker flask and place into the 1.5 mL microfuge tube

8.2.5. Using the 100 µl of cell suspension from microfuge tube from the step above determine cell count and cell viability using automated cell counter

8.3. Subculture of HEK293F cells

Once the culture reaches 1 X 10^6- 3 X 10^6 cells/ml cell density they are subculture into a new flask.

8.3.1. Seed new flask at a cell concentration of 0.3 – 0.6 X 10^6 cells/ml

8.3.2. When density reaches 4 – 6 X10^6 cells/ml subculture flask (3-4 days)

8.3.3. Cryopreserve the cells once the required density is reached or proceed for transfection after 4 passage post resuscitation.

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9. History:

Revision Number	Effective Date	Preparer	Description of Change
0	17AUG2022	Isso Bayala &Hetal Doshi	Initial release