SOP: Transfection and Harvest of Viral Production Cells (VPC) 2.0 -HEK293F for rAAV-CMV-GFP Viral Particle Production

Approvals:

Preparer: Hetal Doshi — Date: 18OCT22
Reviewer: Dr. Maggie Bryans — Date: 21OCT22

1. Purpose:

1.1. This standard operating procedure describes the steps for triple transfection and harvest of Viral Production Cells (VPC) 2.0- HEK293F cells with AAV-DJ Helper Free Expression System for the production of rAAV-CMV-GFP viral particles.

2. Scope and Applicability:

2.1. This SOP can be used to perform the triple transfection and harvest of viral production cells (VPC) 2.0- HEK293 cells for the production of rAAV-CMV-GFP using AAV-DJ Helper free expression system

3. Summary of Method:

3.1. Day 0: Prepare and transfect cells
  3.1.1. Count and dilute cells to get final density of 3 x 10^6 viable cells/ml
  3.1.2. Add AAV-MAX Enhancer to cells
  3.1.3. Prepare plasmid with 1:1:1 ratio of pAAV-GFP vector, pAAV-DJ vector, pHelper vector 45µg total plasmid DNA
  3.1.4. Prepare transfection complexes
  3.1.5. Transfect and incubate for 70-72 hrs.
3.2. Day 3: Harvest of rAAV-CMV-GFP Viral particle
  3.2.1. Add Lysis buffer and Nuclease
  3.2.2. Incubate and centrifuge the cell lysate

4. References:

4.1. 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
4.2. SOP: Labconco Purifier Class II 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 Number: UP22
4.4. SOP: Resuscitation and Culture of Viral Production Cells (VPC) 2.0 -HEK293F, Document No. UP 32
4.5. AAV-DJ Helper Free Expression System by Cell Biolabs, Inc. Catalog number VPK-410-DJ product Manual https://www.cellbiolabs.com/sites/default/files/VPK-410-DJ-aav-helper-free-expression-system.pdf

5. Definitions:

5.1. N/A

SOP: Transfection and Harvest of Viral Production Cells (VPC) 2.0 -HEK293F for rAAV-CMV-GFP Viral Particle Production

6. Precautions:

6.1. Use BSL2 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. 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. Labconco Class II Biological safety cabinet

8.2. Benchmark Scientific Model H3501 Mini Incu-Shaker CO2 with magic clamps for 125ml flasks

8.3. Logos Biosystems Luna-FL Fluorescence Cell Counter

8.4. AAV-MAX Helper-Free AAV Production System Kit, Gibco, Catalog number: A51217

8.5. Viral Production Cells (VPC) 2.0 Gibco catalog #A49784 (HEK293F cells) at 4 to 6 x 10^6cells/ml density and ≥95% viability (three or more passages post-thaw and passage number in the range of 4-25 passages)

8.6. Gibco viral production medium catalog #A4817901

8.7. GlutaMAX™ Supplement 200mM Gibco Catalog #35050061

8.8. Sterile 125-mL PETG Erlenmeyer shaker flask with HDPE Vent Cap, Sterile Chemglass Catalog # CGN-2092-125

8.9. AAV-DJ Helper Free Expression System by Cell Biolabs, Inc. Catalog number VPK-410-DJ (pAAV-DJ Vector, pHelper Vector, pAAV- GFP Control Vector)

8.10. Pierce™ Universal Nuclease for Cell Lysis, Catalog number: 88701

8.11. Pipette aid

8.12. Cryovial rack

8.13. Sterile serological pipettes (2ml, 5ml and 25 ml)

8.14. Lab coat, gloves, sleeves

8.15. 70% Isopropanol

8.16. Sterile cleaning wipes

8.17. 1.5 ml microfuge tube and tube holder

8.18. P20 micropipettes and compatible tips

8.19. 50 ml conical tubes

8.20. 15 ml conical tubes

8.21. Ice in ice bucket

8.22. Sorvall RC 5C Plus centrifuge chilled at 4°C

8.23. Eppendorf centrifuge 5804 R chilled at 4°C

8.24. Nalgene Centrifuge Bottles with Sealing Cap, polypropylene copolymer polypropylene screw closure; silicone gasket; 250 ml Catalog# 3141

SOP: Transfection and Harvest of Viral Production Cells (VPC) 2.0 -HEK293F for rAAV-CMV-GFP Viral Particle Production

9. Procedure:

9.1. **Day 0 Prepare and transfect cells**

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

9.1.2. 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
- 1ml sterile pipettes
- Gibco viral production Medium
- GlutaMax supplement 200mM
- 50ml conical tube
- Pipette aid
- 1.5ml microfuge tube
- Sterile 125mL PETG Erlenmeyer shaker flask with HDPE Vent Cap

9.1.3. Prepare complete growth media by adding 300µl of (100X) GlutaMAX™ Supplement to 29.7ml of viral production medium in a BSC in a 50ml conical tube. Incubate the prepared media at 37°C in a water bath for 10 minutes

9.1.4. Place the 125ml shaker flask with HEK293F cells at a density of 4.5 to 6.0 x 10^6 viable cells/ml and ≥95% viability in the BSC

9.1.5. Aseptically transfer 100µl of cell suspension in a 1.5ml microfuge tube for counting

9.1.6. Place the 125ml Erlenmeyer shaker flask with HEK293F cells in the shaking incubator at 37°C with 8% CO_2 and shaking at 125rpm

9.1.7. Perform the cell count using Logos Biosystems Luna-FL Fluorescence Cell Counter.

9.1.8. Swab the pre-warmed complete growth media prepared in step 9.1.3. with 70% isopropanol and place it in the BSC.

9.1.9. In a new sterile 125ml Erlenmeyer shaker flask aseptically transfer 90 x 10^6 viable cells and add appropriate amount of pre-warmed complete growth media to get final volume of 30ml

9.1.10. Aseptically add 300µl of Enhancer to cell culture flask and incubate the flask at 37° incubator with ≥ 80% relative humidity and 8% CO2 on an orbital shaker at 125 rpm until the DNA/transfection complexation process is completed (steps 9.1.11. through 9.1.17.)

9.1.11. Thaw pAAV-GFP vector, pAAV-DJ vector and pHelper vector on ice

9.1.12. In BSC add 15µg each of pAAV-GFP vector, pAAV-DJ vector, and pHelper vector in a sterile microfuge tube and label plasmid mix

9.1.13. In BSC dilute prepared plasmid mix from step 9.1.12 with Viral-Plex complexation buffer to a final volume of 3ml in a sterile 15ml conical tube. Mix by swirling gently. Label the tube as viral complexation buffer plasmid mix. Store the tube at room temperature till ready to use

9.1.14. Gently invert the AAV-MAX Transfection Reagent bottle 4 to 5 times to mix

SOP: Transfection and Harvest of Viral Production Cells (VPC) 2.0 -HEK293F for rAAV-CMV-GFP Viral Particle Production

9.1.15. In BSC aseptically add 90µl of AAV-MAX Transfection Booster to a new sterile 1.5ml microfuge tube

9.1.16. Add 180µl of AAV-MAX Transfection Reagent to the tube prepared in 9.1.15. Mix by gentle pipetting and label the tube as Transfection complex

9.1.17. In BSC aseptically add entire content from tube labelled transfection complex to the 15 ml conical tube labelled viral complexation buffer plasmid mix prepared in step 9.1.13. Mix by swirling, inverting gently for 3 times. Incubate at room temperature for 30 minutes

9.1.18. In BSC aseptically add prepared plasmid DNA/AAV-MAX transfection Booster/AAV-MAX Transfection reagent complexes from step 9.1.18 to cells prepared in step 9.1.10

9.1.19. Incubate the cells in shaking incubator at 37°C with ≥ 80% relative humidity and 8% CO_2 on an orbital shaker at 125 rpm for 70-72 hrs.

9.2. **Harvest AAV Particles**

9.2.1. 70-72 hrs. post transfection in BSC aseptically add 3.3ml of AAV-MAX Lysis Buffer to the 125ml Erlenmeyer shaker flask containing 293F cells transfected in step 9.1.19

9.2.2. In BSC aseptically transfer 5ml of cell suspension with lysis buffer from step 9.2.1.to a 50ml conical tube labelled HEK 293F cell lysate for qPCR

9.2.3. Aseptically add 12µl of Pierce™ Universal Nuclease to the remaining cell suspension in 125ml Erlenmeyer shaker flask containing HEK293F cells

9.2.4. Incubate the prepared 50 ml conical tube in step 9.2.2 for 1 hr. and 125ml Erlenmeyer shaker flask containing HEK293F cells prepared in step 9.2.3. in the shaking incubator at 37°C with 8% CO2 and shaking at 125rpm for 2 hr.

9.2.5. Centrifuge the 50 ml conical tube labelled HEK 293F cell lysate for qPCR at 4°C at 4,500 x g for 30 minutes in Eppendorf centrifuge 5804 R

9.2.6. In the BSC aliquot 1ml supernatant from 50 ml conical tube to appropriately labeled 1.5ml microfuge tube and store at -80°C for the quantification of AAV titer using qPCR

9.2.7. After 2 hr. incubation of 125ml Erlenmeyer shaker flask containing HEK293F cells, lysis buffer and nuclease prepared in step 9.2.3. aseptically transfer the cell lysate to Nalgene Centrifuge Bottle

9.2.8. Centrifuge the cell lysate at 4,500g for 30 minutes at 4°C in Sorvall RC 5C Plus centrifuge

9.2.9. In BSC use a 25ml pipette to aseptically transfer the supernatant to a 50ml conical tube labelled HEK293F pAAV-GFP cell lysate, date and initial. Store the lysate at 4°C for short duration or at -80°C for further processing.

10. Attachments/Figures

11. History:

Revision Number	Effective Date	Preparer	Description of Change
0	21OCT22	Hetal Doshi	Initial release