SOP: Transduction of CHO K1 cells with Chromatography Purified rAAV GFP Viral Particles

Approvals:
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Reviewer: Dr. Maggie Bryans Date: 25MAY23

1. Purpose:

To describe the steps necessary to perform transduction of CHO K1 cells with affinity chromatography purified rAAV GFP viral particle

2. Scope:

This SOP covers transduction of CHO cells with affinity chromatography purified rAAV GFP viral particle

3. Summary of Method:

3.1. Seeding of CHO K1 cells in six well tissue culture plate
3.2. Transduction of CHO K1 cells with affinity chromatography purified rAAV GFP viral particle
3.3. Cell Lysis using RIPA buffer

4. References:

4.1. CHO K1 Cells Product Sheet, https://www.atcc.org/products/ccl-61
4.2. Viral Particle AAV2/DJ-CMV-GFP User Manual for Ready-to-Use AAV product, Vector Biolabs
4.3. RIPA Lysis and Extraction Buffer User Guide, Thermo Scientific
4.4. SOP: Operation of Logos biosystems Luna-FL Fluorescence Cell Counter for Fluorescence Cell Counting Document Number: UP22
4.5. SOP: Labconco Purifier Class 2 Biological safety Cabinet (BSC) Operation
4.6. SOP: Transfection and Harvest of Viral Production Cells (VPC) 2.0 -HEK293F for rAAV-CMV-GFP Viral Particle Production Document Number: UP33
4.7. SOP: Isolation of AAV-GFP Viral Particle from HEK293F AAV GFP Cell Lysate by Affinity Chromatography on the ÄKTA pure Chromatography System

5. Definitions:

5.1. Multiplicity of infection = AAV genome copies to be used per # of cells to be infected
5.2. Physical titer is a measurement of how much virus is present, and is expressed as the number of viral particles per mL (VP/mL), or for AAV as genome copies per mL (GC/mL)

6. Precautions:

6.1. Use BSL1 safety measures 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 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 Purifier Class 2 Biological safety cabinet

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8.2. Individually wrap sterile Serological Pipettes (1ml, 2ml, 5ml, 10 ml and 25ml)
8.3. Pipette aid
8.4. 6 Well Cell Culture Plate Corning costar REF 3516
8.5. EVOS XL Core Imaging System
8.6. EVOS Fl digital microscope system
8.7. Incubator with 5% CO_2
8.8. Luna Fl Fluorescence cell counter, counting chamber and Acridine orange/propidium Iodide stain.
8.9. 15 ml conical tube
8.10. Conical tube rack
8.11. 1.5 ml Eppendorf tubes
8.12. microfuge tube rack
8.13. One T75 tissue culture flask of CHO K1 cells with 90% confluency for seeding one 6 well plate.
8.14. Affinity chromatography purified rAAV GFP viral particle
8.15. Ham's F12 + 10% FBS CHO K1 complete growth media
8.16. PBS
8.17. Trypsin
8.18. RIPA Lysis and Extraction Buffer, Catalog number: 89900
8.19. Halt cocktail (protease inhibitor) Thermo Scientific™ Halt™ Protease Inhibitor Cocktail, EDTA-Free (100X) catalog # 78425
8.20. Ice in an ice bucket
8.21. Eppendorf centrifuge 5424R centrifuge

9. Procedure:

9.1. Day 1: Seeding of a 6 well cell culture plate

9.1.1. Pre warm CHO K1 complete growth media, PBS and trypsin in a bead bath set at 37°C for 15 minutes
9.1.2. Prepare the biological safety cabinet as per the operation SOP.
9.1.3. Spray the following items with 70% ethanol and place it in BSC.

- Prewarmed CHO K1 media
- PBS
- Trypsin
- Pipette aid
- Serological pipettes (1ml, 2ml,5ml.10 ml, 25 ml)
- 1.5 ml Eppendorf tube
- 15 ml conical tube
- 1.5ml tube rack and conical tube rack
- 6 well cell culture plate

9.1.4. Observe the 1 T75 flask of CHO K1 cells for visual contamination.
9.1.5. Observe the flask under EVOS core imaging system and record your observation.
9.1.6. Place the flask in the BSC

9.1.7. Aseptically remove the spent media
9.1.8. Aseptically add 7 ml of 1X PBS away from the monolayer of the cells
9.1.9. Wash the monolayer of cells with 1X PBS by rocking back and forth gently.
9.1.10. Aseptically remove 1X PBS
9.1.11. Aseptically add 3ml of trypsin
9.1.12. Lay the flask horizontally on the surface and allow trypsin to react for 2 minutes.
9.1.13. Observe the cells for dislodgment under the microscope. If cells are not dislodged give a tap on the narrow side of the flask
9.1.14. Observe the cells for dislodgment under the microscope.
9.1.15. Quickly in the BSC neutralize the cells by aseptically adding 7 ml of CHO K1 complete growth media.
9.1.16. Pipette up and down without forming bubbles to get a single cell suspension.
9.1.17. Aseptically transfer 200 µl of cell suspension to 1.5 ml Eppendorf tube.
9.1.18. Aseptically transfer remaining cell suspension to a 15 ml conical tube
9.1.19. Centrifuge the conical tube with cells at 120 X g for 5 minutes. **Note:** Balance the cell suspension tube with a 15 ml conical tube containing same volume of water.
9.1.20. Meanwhile count the cells by following the SOP: Operation of Logos biosystems Luna-FL Fluorescence Cell Counter for Fluorescence Cell Counting Document Number: UP22
9.1.21. Resuspend the cells in the appropriate volume of CHO K1 complete growth media to get a cell density of 250,000 cells/ml
9.1.22. Add 1ml of cell suspension from step 9.1.21 to each well of six well plate.
9.1.23. Add 2ml of prewarmed CHO K1 complete growth media to each well of six well plate.
9.1.24. Incubate the plate at 37°C with 5 % CO_2 for about 20 hours.

9.2. Day 2: Infection of CHO K1 cells with affinity chromatography purified rAAV GFP viral particle

9.2.1. Before beginning, make sure the cells per well on the 6 well plate is at least 50% confluent.
9.2.2. Prepare the BSC as per operation SOP
9.2.3. Spray the following items with 70% ethanol and place it in BSC:

- Pre-warmed CHO K1 media
- Affinity chromatography purified rAAV GFP viral particle
- Pipette aid
- Serological pipettes (1ml, 2ml,5ml.10 ml)
- 1.5 ml Eppendorf tube
- 5 15 ml conical tube
- Conical tube rack
- 1X PBS
- Micropipettes p1000 and p20 and micropipette tips for p1000 and p20

9.2.4. Thaw the affinity chromatography purified rAAV GFP viral vector on ice.

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9.2.5. In BSC label three 15 ml conical tubes “1”, “2”, and “3”, respectively. Transfer 2 ml of pre-warmed CHO K1 media to each tube.
9.2.6. Add purified rAAV GFP viral particles to the prepared conical tubes with 2 ml pre-warmed CHO K1 media as below:

Tube	Amount of rAAV GFP Viral Particles
Tube 1	0 µl
Tube 2	10 µl
Tube 3	50 µl

9.2.7. Place the 6 well plate with CHO K1 cells in the BSC.
9.2.8. Label the six well plate on the side as “Transduction’ and with the date and your initials.
9.2.9. Label the lid of each well of the 6 well plate as listed in the table below:

Tube 1 (-)	Tube 2	Tube 3
Tube 1 (-)	Tube 2	Tube 3

9.2.10. Aseptically aspirate spent media with a Pasteur pipette attached to the vacuum pump in BSC from all 6 wells.
9.2.11. Add 1ml of media/media with virus prepared in step 9.2.6 to the appropriate well (see 9.2.9)
9.2.12. Incubate the plate at 37°C with 5% CO_2
9.2.13. After 24 h of incubation observe the cells under the inverted microscope and fluorescent microscope. In the BSC aseptically remove spent media and add 1ml of prewarmed CHO K1 media to each of the six well.
9.2.14. Incubate the plate for another 24 h before harvesting for further analysis. Observe the cells under inverted microscope and image the cells using the fluorescent microscope.

9.3. Preparation of cell lysate for SDS-PAGE and Western Blot
9.3.1. After 48h total incubation harvest cells
9.3.2. Pre-chill the centrifuge at 4°C
9.3.3. Prepare the BSC as per operation SOP.
9.3.4. Spray the following items with 70% ethanol and place it in BSC.

- Pipette aid
- 1.5 ml Eppendorf tube
- microfuge tube rack and conical tube rack
- RIPA Buffer
- Halt cocktail
- Micropipettes p1000 and p20 and micropipette tips for p1000 and p20

9.3.5. In BSC aseptically add 13µl of Halt cocktail to 1300µl of RIPA buffer. Keep in on ice

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9.3.6. Place the 6 well plate labelled "Transduction" in the BSC. Aspirate the spent media with a Pasteur pipette connected to the vacuum pump being careful not to disturb the cells from all the wells.
9.3.7. Wash the cells by adding 2ml of ice cold 1X PBS to the inside of each well without disturbing the cells. Aspirate the PBS.
9.3.8. Repeat step 9.3.7.
9.3.9. Add 200µl of ice-cold RIPA buffer and halt cocktail mix prepared in step 9.3.5. to all the wells
9.3.10. Incubate the plate on ice for 5 minutes, swirl the plate every 2 minutes to ensure uniform coverage.
9.3.11. Using a small scrapper dislodge the cells and collect the cell lysate on one side of the well, using a micropipette transfer the cell lysate to a sterile 1.5ml microfuge tube labelled appropriately. Repeat this step for all the wells.
9.3.12. Centrifuge the cell lysate in the prechilled microcentrifuge ~14,000 × *g* for 15 minutes.
9.3.13. In the BSC carefully transfer the supernatant to a sterile appropriately labelled 1.5ml microfuge tube
9.3.14. Store the cell lysate at 4°C for short term or at -20°C for long term storage.

10. Attachments/Figures

11. History:

Revision Number	Effective Date	Preparer	Description of Change
0	17MARCH2023	Hetal Doshi	Initial release