Jul 11, 2024

Adeno-associated virus (AAV) production and administration

DOI
dx.doi.org/10.17504/protocols.io.bp2l623b5gqe/v1

Shiyi Wang[1]

[1]Duke University

ASAP Collaborative Rese…

Shiyi Wang
Duke University

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.bp2l623b5gqe/v1

Protocol Citation: Shiyi Wang 2024. Adeno-associated virus (AAV) production and administration. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.bp2l623b5gqe/v1**

Protocol status: Working
We use this protocol and it's working

Created: July 11, 2024

Last Modified: July 11, 2024

Protocol Integer ID: 103196

Keywords: ASAPCRN

Funders Acknowledgement:
Aligning Science Across Parkinson's (ASAP) initiative
Grant ID: ASAP-020607

Disclaimer

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to **protocols.io** is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with **protocols.io**, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

Abstract

Adeno-associated virus (AAV) production and administration

1 **Transfection of HEK293T Cells**

1.1 - Transfect HEK293T cells with the following plasmids: pAd-DELTA F6, serotype plasmid AAV PHP.eB, and AAV plasmid (pZac2.1-GfaABC1D-Ezrin-BioID2-HA or pZac2.1-GfaABC1D-Ezrin T567A-BioID2-HA).

2 **Cell Lysis and AAV Purification**

2.1 - Three days after transfection, collect cells in a lysis buffer (15 mM NaCl, 5 mM Tris-HCl, pH 8.5).

2.2 - Perform repeat freeze-thaw cycles followed by treatment with Benzonase at 37°C for 30 minutes.

2.3 - Centrifuge lysed cells to pellet debris, and collect the supernatant containing AAVs.

3 **Optiprep Density Gradient Centrifugation**

3.1 - Apply the supernatant containing AAVs to an Optiprep density gradient (15%, 25%, 40%, and 60% iodixanol).

3.2 - Centrifuge at 67,000 rpm using a Beckman Ti-70 rotor for 1 hour.

3.3 - Collect the AAV-enriched fraction from between the 40% and 60% iodixanol layers.

4 **AAV Concentration**

4.1 - Concentrate the AAV fraction by repeated washes with sterile PBS using an Amicon Ultra-15 filtration unit (100 kDa NMWL) to a final volume of approximately 100 µl.

4.2 - Aliquot the concentrated AAVs for storage at −80°C.

5 **Mouse Anesthesia and Surgery**

5.1 - Anesthetize 9-week-old WT or LRRK2 G2019Ski/ki mice using 1.5% isoflurane gas in a stereotaxic frame.

6 **Intravenous AAV Injection**

6.1 - Inject 10 µl of purified AAVs (titer of ~1 x 10^12 GC/ml) into the mouse brain intravenously by injection into the retro-orbital sinus.

7 **Post-Injection Care and Tissue Preparation**

7.1 - Allow mice to recover for 3 weeks until they reach 12 weeks of age.

7.2 - Anesthetize mice with 200 mg/kg Tribromoethanol (Avertin) and perform transcardial perfusion with TBS/Heparin followed by 4% paraformaldehyde (PFA) at room temperature (RT).

8 **Brain Processing**

8.1 - Post-fix harvested brains overnight in 4% PFA, then cryoprotect in 30% sucrose.

8.2 - Embed brain blocks in O.C.T. (TissueTek) and store at −80°C.

9 **Cryosectioning**

9.1 - Cut 30 µm thick brain sections using a Leica CM3050S vibratome.

9.2 - Store sections in a mixture of TBS and glycerol at −20°C for subsequent antibody staining procedures.

10 Notes:

10.1 - Ensure all procedures are performed in accordance with institutional guidelines for animal care and use.

10.2 - Maintain sterile conditions during AAV preparation and injection procedures to prevent contamination.

10.3 - Document all experimental details including AAV titers, injection volumes, and storage conditions for reproducibility.