Jul 10, 2024

Adeno-associated virus (AAV) production and administration

DOI
dx.doi.org/10.17504/protocols.io.8epv5jyynl1b/v1

Shiyi Wang[1]

[1]Duke University

ASAP Collaborative Rese…

Shiyi Wang
Duke University

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.8epv5jyynl1b/v1

Document Citation: Shiyi Wang 2024. Adeno-associated virus (AAV) production and administration. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.8epv5jyynl1b/v1**

License: This is an open access document distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Created: May 23, 2023

Last Modified: July 10, 2024

Document Integer ID: 82329

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and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to **protocols.io** is not peer reviewed and may not have undergone a formal approval of any kind.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. HEK293T cells were transfected with 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. Three days after transfection, cells were collected in 15 mM NaCl, 5 mM Tris-HCl, pH 8.5, and lysed with repeat freeze-thaw cycles followed by treatment with Benzonase (Novagen 70664) at 37°C for 30 minutes.

3. Lysed cells were pelleted by centrifugation, and the supernatant, containing AAVs, was applied to an Optiprep density gradient (Sigma D1556, 15%, 25%, 40%, and 60%) and centrifuged at 67,000 rpm using a Beckman Ti-70 rotor for 1 hour.

4. The AAV-enriched fraction was isolated from between 40% and 60% iodixanol solution and concentrated by repeated washes with sterile PBS in an Amicon Ultra-15 filtration unit (NMWL: 100 kDa, Millipore UFC910008) to a final volume of ~100 µl and aliquoted for storage at −80°C.

5. 9-week-old WT or LRRK2 G2019S$^{ki/ki}$mice placed in a stereotaxic frame were anesthetized through inhalation of 1.5% isofluorane gas.

6. 10 µl of purified AAVs having a titer of ~1 × 10^{12}GC/ml was introduced into the mouse brain intravenously by injection into the retro-orbital sinus.

7. After 3 weeks at 12-week-old, mice were anesthetized with 200 mg/kg Tribromoethanol (Avertin) and transcardially perfused with TBS/Heparin and 4% paraformaldehyde (PFA) at room temperature (RT).

8. Harvested brains were post-fixed overnight in 4% PFA, cryoprotected in 30% sucrose, and the brain blocks were prepared with O.C.T. (TissueTek) to store at −80°C.

9. 30 µm thick brain sections were obtained through cryosectioning using a Leica CM3050S (Leica, Germany) vibratome and stored in a mixture of TBS and glycerol at −20°C for further free-float antibody staining procedures.