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Adeno-associated virus (AAV) production and administration

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We use this protocol and it's working

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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 $\sim 1 \times 10^{12}$ 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.