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Intravenous (tail vein) injection of AAV into rat

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We use this protocol and it's working

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Abstract

This protocol is used to administer adeno-associated virus (AAV) in male and female rats. This procedure is performed under anesthesia and should incorporate all local requirements for standards of animal experimentation, including methods of anesthesia, surgical environment, and post-operative monitoring and care.

Guidelines

All containers that are in contact with virus waste need to be properly labeled as biohazard waste, and disinfected appropriately following institutional safety requirements.
Virus stock needs to be placed on ice during surgeries.
For virus transport make sure to follow institutional safety requirements, e.g., double-containers are required for safe transport of biohazards (e.g. Eppendorf tube placed into 50ml Falcon tube).

Materials

- Isoflurane **Zoetis Catalog #**10015516
- 1 ml syringes (or U-100 Insulin Syringe) **BD Biosciences Catalog #**329461
- 0.9% Saline Solution **Thermo Fisher Catalog #**BO0334B
- 70% Ethanol
- 10% Bleach
- AAV-PHP.S:hSyn1-tdTomato **addgene**
- Sterile gauze pads (1 pad per patient) **Contributed by users**
- Biohazard disposal container **Contributed by users**
- Virkon™ S 5O tablets/bottle **Fisher Scientific Catalog #**NC9821357

Preparation

1 Determine the dose of virus to administer per rat. Divide the dose (viral genomes (vg)) by the titre (vg/ml) to calculate the volume of virus needed to inject one rat.

Viral dosage can be determined using: AAV-viraldosage-calculations.xlsx

2 In a screw-cap vial, prepare the solution to be administered. Add virus, and dilute with sterile 0.9% saline to a total 150 µl/rat. Briefly vortex each vial for 1 - 2 s before use. Transport the virus on ice once it is ready for injection.

Surgery

3 Anesthetise animal (2.5% isoflurane in oxygen, or as required for maintenance).

4 While the rat is being induced, load a U-100 insulin syringe with virus. Remove the dead space in the syringe barrel by gently ejecting the virus back into the tube such that air bubbles are expelled. Load the syringe again and repeat the procedure until no bubbles remain in the barrel.

5 Place animal on heated pad.

6 Apply 70% Ethanol wipes to tail and turn 45º for a visible presentation of tail vein. If unable to visualise the vein, warm the area.

7 Introduce a U-100 insulin syringe into the tail vein. Withdraw the barrel slightly to confirm location in the vein. Gently inject the virus.

8 Apply light pressure over the injection site for approximately 30 s to prevent backflow.

9 Return rat to housing cage and monitor for any evidence of swelling or complications at the injection site. Continue to monitor over the period of incubation for adverse reactions.

Tissue Harvesting

10 Anesthetise and fix animals by intra-cardiac perfusion, then remove tissues of interest for further study.