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Bilateral Adeno-associated virus (AAV) injection into interscapular brown adipose tissue

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1 Works for me



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ABSTRACT

Protocol details how to inject AAV virus into each interscapular brown adipose tissue fat pad in the mouse.

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Jun 30, 2020

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38739

GUIDELINES

All containers that are in contact with virus waste need to be properly labeled as biohazard waste.

Virus stock needs to be placed on ice during surgeries.

For virus transport make sure to follow IBC safety rules, double-containers are required for safe transport of biohazards (e.g. Eppendorf tube placed into 50ml Falcon tube).

MATERIALS TEXT

MATERIALS

☒ Kimtech Science™ Kimwipes™ Delicate Task Wipers, 1-Ply Thermo

Fisher Catalog #066661A

☒ Surgical suite and animal housing Contributed by users

☒ Surgery cards and surgery record Contributed by users

☒ Personal protective equipment (PPE): lab coat gloves face mask Contributed by users

☒ Eye lubricant Contributed by users

☒ Timer Contributed by users

☒ Biohazard stickers Contributed by users

☒ Hair clippers Contributed by users

☒ Hamilton® syringe 700 series fixed needle 702N 25 uL Sigma

Aldrich Catalog #20735

☒ Aluminum foil Contributed by users

☒ Biohazard waste container Contributed by users

☒ Sterile towels Contributed by users

☒ Surgery tools (scissors hemostats tissue forceps toothed forceps) Contributed by users

☒ Sterile wound clip remover Contributed by users

☒ Sterile wound clips Contributed by users

☒ Sterile cotton swabs and gauze Contributed by users

☒ Isoflurane vaporizer oxygen tanks and anesthesia induction chamber Contributed by users

☒ Glass bead sterilizer Contributed by users

☒ (2) Heating mats Contributed by users

☒ Adeno-associated virus (rAAV6/hSyn-mCherry-cre) Contributed by users

☒ Portable centrifuge Contributed by users

☒ Bupivacaine/lidocaine Contributed by users

☒ Carprofen for mice Contributed by users

☒ Sterile saline Contributed by users

☒ Novalsan Contributed by users

☒ 70% isopropyl alcohol Contributed by users

☒ 70% ethanol Contributed by users

☒ Sterile distilled water Contributed by users

☒ 0.5 mL syringes for bupivacaine/lidocaine caprofen Contributed by users

☒ 3 mL syringes for saline Contributed by users

☒ 70% ethanol spray bottle Contributed by users

BEFORE STARTING

Approval of Institutional Biosafety Committee (IBC) to work with AAV

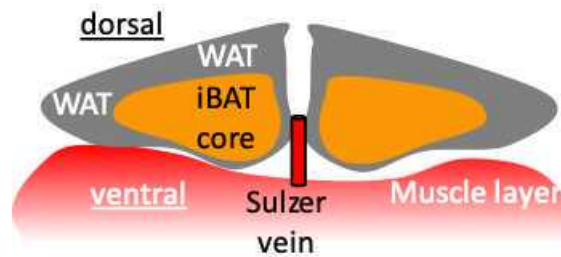
Approval of Institutional Animal Care and Use Committee (IACUC) to perform AAV injections in animals

Surgery

- 1 Place a sterile towel on the surgery platform over the heating mat, another sterile towel in the anesthesia induction chamber.
- 2 Record the mouse's body weight to prepare syringes for saline and analgesia treatment.

Warm saline (1-1.5 mL for 25 g body weight); bupivacaine/lidocaine (2.5-12.5 mg/kg body weight); carprofen (5-10 mg/kg body weight).
- 3 Induce anesthesia with 5% isoflurane in induction chamber.
- 4 Place the mouse on the surgery platform and maintain anesthesia via a nose cone at 1.5-2% Isoflurane/oxygen (adjust for each mouse accordingly to breathing and anesthetic depth (e.g. loss of withdrawal reflex)).
- 5 Apply eye lubricant to the mouse's eyes.
- 6 Disinfect the lower back of the mouse with 70% ethanol, then administer subcutaneous carprofen injection in the disinfected area.
- 7 Check for full withdrawal reflex to ensure anesthetic depth.
- 8 Use clippers to shave the interscapular region of the mouse and remove all hair.
- 9 Disinfect the skin by three alternating scrubs of Novalsan followed by 70% Isopropyl rubbing alcohol.
- 10 Make a midline incision with sharp scissors from the scapula to the neck.
- 11 Remove connective tissue underneath the skin incision using a sterile cotton swab and toothed forceps until the interscapular brown adipose tissue (IBAT) depot is fully accessible.

- 11.1 Note: The iBAT pad (light brown color) is surrounded by a layer of white adipose tissue. We have successfully injected virus dorsally into the iBAT, however, we could increase our PRV infection success rate by placing the injections into the iBAT core and accessing the ventral iBAT site. The rostral portion of the iBAT depot is detached from the interscapular muscle and the iBAT lobe is flipped back from the rostral side. This will allow visibility of the main iBAT vein (Sulzer vein) and injections are placed from



the ventral iBAT side (see Figure 1).

Figure 1: Schematic of iBAT depot.

- 12 Cover the iBAT with a saline soaked sterile gauze to keep the tissue from drying, then prepare the Hamilton syringe for injection.

12.1

AAV6 is considered a BSL 1 pathogen, therefore viruses do not require preparation under a laboratory fume hood. However, to ensure aseptic technique, we line an area of the lab bench with aluminum foil and prepare the virus over the aluminum foil. Once surgery is complete, aluminum foil is disposed of in biohazard and area is sanitized.

- 13 Fill the Hamilton syringe with 25 μ L, return to the surgery platform with the Hamilton syringe to begin the injections.
- 14 Remove the saline-soaked gauze from the brown adipose tissue and locate the brown adipose tissue pad.
- 15 Administer (5) 2.5 μ L injections into the right iBAT pad and (5) 2.5 μ L injections into the left iBAT pad. Hold the syringe in place for ~30 seconds after each injection to minimize backflow, slowly pull the syringe out of the BAT while holding a sterile cotton swab on the site of injection to dry of any possible virus leakage.
- 16 Place a sterile, saline-soaked gauze on top of the brown adipose tissue and clean the syringe over aluminum foil. Flush the syringe 5x with 70% EtOH, then flush 5x with distilled H₂O.

16.1 Collect all liquid as biohazard waste.

- 17 Remove the saline-soaked gauze from the brown adipose tissue and with a sterile cotton swab and toothed forceps, reposition the overlying white adipose tissue back to its original location prior to your incision.

- 18 With the teether and tissue forceps align both sides of the incision properly, and apply wound clips (~25 mm apart, to ensure proper blood circulation).
- 19 Inject bupivacaine/lidocaine subcutaneously around the incision site.
- 20 Remove mouse from bite bar and administer intraperitoneal injection of warm saline for rehydration.
- 21 Place the unconscious mouse in a fresh cage with one cage side on a heating mat and monitor until startling reflexes are restored.
- 22 Replenish all consumables (sterile cotton swabs, sterile gauze) and use 70% ethanol to clean surgical tools and place in glass bead sterilizer for 15-20 seconds before additional surgeries.