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Protocol status: In development
 We are still developing and optimizing this protocol

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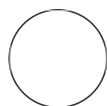
Intrapancreatic injection surgery

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ABSTRACT

This is a protocol for performing intrapancreatic injection of Cholera Toxin Beta (CTb) in mice.

MATERIALS

MATERIALS



1 g Lidocaine biorbyt Catalog
 #orb341946



100 g Povidone Iodine biorbyt Catalog
 #orb322666



Buprenorphin Contributed by users Catalog #961425



Isoflurane (Forane) Baxter Catalog #1001936040



Cholera Toxin Subunit B (Recombinant) Alexa Fluor™ 488
 Conjugate Thermofisher Catalog #C34775

SAFETY WARNINGS



Wear Protective Personal Equipment (masks, labcoat, gloves) when working with animals. Isoflurane is an eye and skin irritant and central nervous system. Always include a proper scavenging system when utilizing it.







BEFORE START INSTRUCTIONS

- This study was approved by the Institutional Animal Care and Use Committee of Icahn School of Medicine at Mount Sinai
- Clean and sterilize properly all surgical tools needed.

Preparing surgery area

- 1
 - Prepare the surgical space by placing the absorbant pad and the inhalatory anesthesia. Write down the mouse weight.
 - Anesthetize the animal by inducing anesthesia with 3% Isoflurane. Once anesthesia is induced, place animal in supine position and keep anesthesia at 1-1.5%.
 - Apply eye lubricant (eg. Lacri Lube). Subsequently apply hair removal cream (eg. Nair) in the area of incision (left abdominal area). Apply Povidone/Yodine in the incision area. Apply Lidocaine afterwards. Apply Ethanol swab. Now the surgical area is ready for incision

Surgery

- 2
 - Make a vertical incision of 1 to 1.5cm, left side of the abdomen. Gently cut first skin, then abdominal wall. Cut a piece of gauze and wet it in sterile PBS, place close to the incision point. With the help of cotton tips or arruga forceps, expose the pancreas and place it in the gauze.
 -  100 µg of CTb is dissolved in  5 µL Sterile PBS. With the use of a  5 µL Hamilton syringe, perform 5 injections of  1 µL volume each. In the case of AAV injection, total volume of injection should range between  5 µL -  20 µL, depending on the concentration needed. Inject in several places of the pancreas (spread between head and tail of the pancreas). Every injection should be performed at a slow pace (5-10 seconds), waiting 10-15 seconds before removing syringe.
 - Place the pancreas back to the abdominal cavity. Suture (2-3 sutures) the abdominal wall, using Vicryl Suture 4-0, 1/2c RB1 (or similar). Close the skin with an autoclip applier.

Post-surgery care

- 3
 - After surgery, inject Buprenorphin (SC, 0.05-0.1 mg/kg) and place animal back to its cage. Analgesia should be injected the 2 following days, twice a day. Monitor animal weight and behavior.

CTb expression

- 4
 - Harvest tissues of interest: Coeliac ganglia, Nodose ganglia, Dorsal Root Ganglia 1 week after injection of CTb. In the case of AAVs, harvest tissues 3-4 weeks after injection.

