

**Retro-Orbital Injection in Mice**  
**IACUC Standard Procedure**  
**Effective Date: February 2023**



**Description of procedure:**

The retro-orbital injection is an acceptable alternative to the tail vein injection route, with proper training. The route designated by the protocol should therefore be based on the researcher's level of training and proficiency with the retro-orbital or tail vein injection.

When administering a retro-orbital injection, anesthesia is required in animals  $\geq 7$  days of age. When performed proficiently, this procedure will take less than five minutes. In which case, a heat source is not required.

Up to 1% of the animal's body weight in volume can be administered per injection. The maximum injectable volume  $< 200\mu\text{L}$  in adults and  $10\mu\text{L}$  in neonates. A mouse can receive no more than one injection or retro-orbital sample collection per day. When more than RO procedure is required, alternate between eyes and allow 1-2 days between procedures on the same eye. Do not exceed two injections per eye in a mouse.

**Juvenile and adult mice ( $\geq 7$  d old)**

Supplies:

- Inhalant Anesthesia (Isoflurane) preferred. Ketamine/Xylazine as injectable if inhalant is not available.
  - Gloves
  - 0.5-1ml syringes with a needle size (27-29-gauge) not to exceed 1/2" in length to avoid trauma.
  - Gauze sponges
  - (Optional) ophthalmic anesthetic (0.5% proparacaine hydrochloride ophthalmic solution, Alcon Laboratories, Inc., Fort Worth, TX)
1. Anesthetize the mouse.
  2. Position the mouse on its side. Restrain it with the thumb and middle finger of the non-dominant hand, pulling back the loose skin over the shoulders and behind the ears, making sure not to put pressure on the neck/trachea.
  3. Use the index finger of the non-dominant hand to draw back the skin above the eye and the thumb to draw back the skin below the eye. The eye should protrude slightly.
  4. (Optional) Place a drop of ophthalmic anesthetic (0.5% proparacaine hydrochloride ophthalmic solution) on the eye that will receive the injection. This provides additional procedural and post-procedural analgesia.
  5. Insert 1/4 to 1/3 of the needle, **bevel down**, at an angle of approximately  $45^\circ$ , through the medial canthus (3 o'clock position) or the inferior fornix conjunctival membrane (6 o'clock position into the eye socket). There is a degree of resistance, which causes the eye to retreat back into the sinus, until the needle pierces through the conjunctiva. The needle will be positioned behind the globe of the eye in the retro-bulbar sinus. A sharp cutting needle is preferred as it results in reduced tissue distortion and damage.
  6. Inject slowly into the retro-bulbar sinus. Do not aspirate before injection, as this will collapse the vessels
  7. Remove needle gently to prevent injury to the eye.
  8. Close the eyelid and apply mild pressure to the injection site with a gauze sponge.
  9. Monitor the mouse during the recovery process.
  10. Examine the injection site for swelling or other visible trauma.
  11. If no adverse effects are observed, return the mouse to its home cage, once it regains its righting reflex.

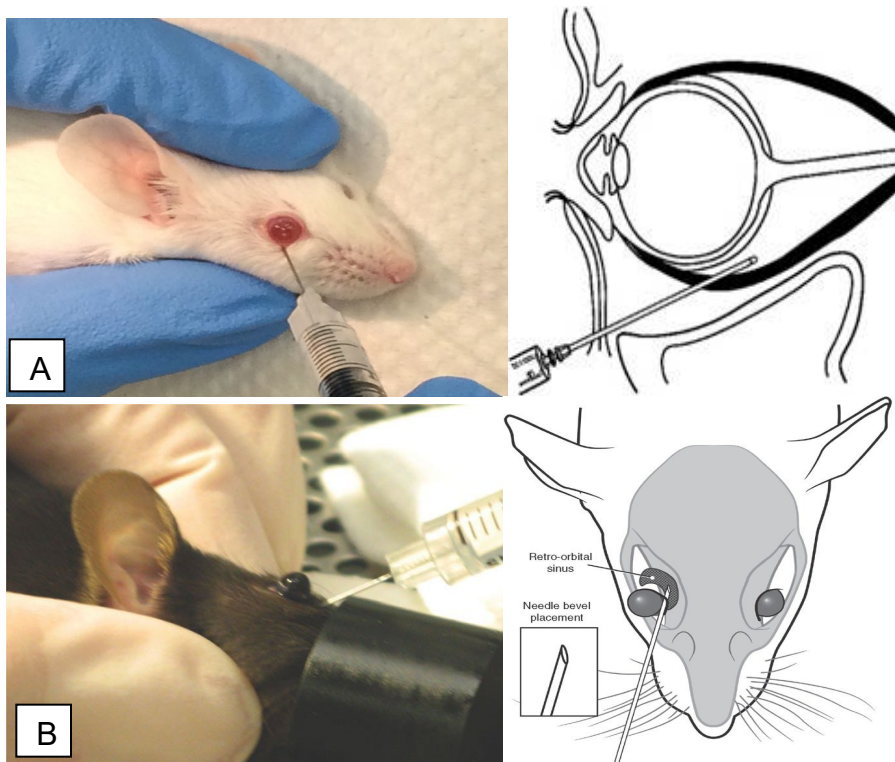


Figure 1. Insert needle, bevel down, at the inferior fornix (A) or medial canthus (B), into the retro-orbital sinus.

#### NEONATES ( $\leq 6$ d old):

Pups should be separated from their mothers as they may emit stress vocalizations during the procedure. Pups are also susceptible to hypothermia and need to be supplied heat once removed from their home cage. Pups should be contained, on indirect heat, covered with gauze to provide added warmth. Hypothermia cryoanesthesia is an option for 3-6-day old animals for optimal immobilization, but should not be needed for animals 1-2 days old. See [IACUC Standard Procedure for Hypothermia Cryoanesthesia in Neonatal Rodents](#) for more details.

#### Supplies:

- Gloves
- Smaller syringes such as 0.5 mL may provide optimal volume for delivery
- Needle (31-gauge, not to exceed 0.3125" in length to avoid trauma)
- Gauze sponges
- Indirect heat source (e.g., heat pack covered with paper towel)

1. Remove the mouse from the holding cage and position the mouse on its side. Gently restrain it with the thumb and forefinger of the non-dominant hand.
2. Insert approximately 1/4-1/3 of the needle length, bevel down, at a 45° angle in the area that will become the medial canthus (3 o'clock position into the eye socket). The needle will be positioned behind the globe of the eye in the retro-bulbar sinus. A sharp cutting needle is preferred as it results in reduced tissue distortion and damage.
3. Inject into the retro-bulbar sinus.
4. Remove needle gently to avoid injury to the eye.
5. Apply mild pressure to the injection site with a gauze sponge.

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**Agents:** This procedure requires inhalant (isoflurane) or injectable (Ketamine/Xylazine) anesthesia for juveniles and adults. Hypothermia cryoanesthesia is an option for 3-6-day old neonates. All agents administered to animals must be listed in the "Agents" section of the approved IACUC protocol.

**Adverse Effects:** Swelling around injection site, proptosis, eye trauma, blindness, or localized tumor growth.

**References:**

[Yardeni T, Eckhaus M, Morris D, Huizing M, Hoogstraten-Miller S. Retro-orbital injection in mice. \*Lab Anim \(NY\)\* 2011 May; 155-160.](#)