

**The following policy must be followed for all procedures:**

- UCSF IACUC [Guidelines for Rodent Anesthesia](#)

**Description of procedure:**

**General:** Hydrodynamic delivery is an effective method for intracellular delivery of substances in rodents. This technique relies on the force generated by the rapid injection of a large volume of solution to increase the permeability of cell membranes of perfused organs. The procedure was initially established for gene delivery in mice and its applications have been extended to the delivery of proteins, DNA and RNA sequences, and small molecules.

**Objective:**

- Provide guidance on performing hydrodynamic tail vein delivery of substances in mice.

**The protocol must identify:**

- Anesthesia used for the procedure or if performed awake in a restraint.
- If not using anesthesia, a justification and approval by the IACUC is required, and animals will be placed under USDA category E.
- If given multiple doses, a justification and approval from the IACUC is *required*, and the time interval between the doses should be no less than 5 days.

**Preparation:**

1. Allow all solutions to reach room temperature prior to injection.
2. Weigh mice and determine the amount of saline (0.9% sodium chloride) needed by assuming 10% of body weight for the injection of each mouse.
3. For each mouse, fill a sterile 3 ml syringe and ensure to avoid air bubbles.
4. Switch to a new, sterile 27-gauge needle and fill the needle and hub with liquid completely without introducing air bubbles.
5. Warm the mice to dilate blood vessels by placing the mouse cage on a heat pad or under a heat lamp for 5 min. Mice should be carefully monitored for signs of overheating (increased respiration, decreased activity).

**Hydrodynamic Injection:**

1. Lightly anesthetize the mouse with isoflurane gas.
  - a. If no anesthesia is used, transfer mouse into the barrel of the plastic or brass restrainer, which restrains the animal while allowing access to the tail vein.
2. Position the mouse so one of the lateral tail veins is visible. Wipe the tail of the mouse with an alcohol swab.
3. Pull the tail taut, position the syringe parallel to the tail with the needle pointing toward the body of the mouse and place the needle, bevel up, approximately 30° from the plane of the tail. It is recommended to first inject in the distal half of the tail so that should the initial injection be unsuccessful, the needle can be repositioned closer towards the trunk of the mouse

Proceed with the injection only if the needle is correctly inserted, which is evident from the needle sliding in with no resistance.

- a. If the needle is not in the vein, the tissue near the injection site will change color, become swollen and the plunger will not move easily. In that case, halt the injection, wait 2-3 min and try again at a site closer to the tail base.
4. Press down on plunger in one, continuous motion and inject the full volume of liquid in 5-10 seconds.
5. Remove needle from the vein and stop the bleeding by applying gentle pressure to the tail. Hold onto the tail of the mouse until bleeding stops completely.

#### **Post-procedure monitoring and recovery:**

1. Place animal into a recovery cage placed half on top of an appropriate heating source.
2. An initial period of panting and immobility is normal, however mice usually show signs of recovery in approximately 5 minutes.
3. If anesthetized, recover per UCSF IACUC [Guidelines for Rodent Anesthesia](#)
4. Observe animals until their respiration and activity level are resumed.

#### **Literature search words required:**

Literature search was performed for refinement of this Standard Procedure in 2022:

<b>Key Words</b>	<b>Search Site</b>	<b><u>Years Covered</u></b>
<i>rodent hydrodynamic transfection/injection/delivery</i>	Pubmed and Google Scholar	2010-2022
<i>Rodent hydrodynamic injection with/without anesthesia</i>	Pubmed and Google Scholar	2010-2022

**Agents:** This procedure requires anesthetics and analgesics. All agents administered to animals should be listed in the “Agents” section of RIO.

**Adverse Effects to be considered:** Immediate after the injection mice may have reduced activity or labored breathing for about 5 minutes. Blood loss, peri-vascular irritation at injection site.

#### **References:**

Bonamassa, Barbara et al. “Hydrodynamic gene delivery and its applications in pharmaceutical research.” *Pharmaceutical research* vol. 28,4 (2011): 694-701. doi:10.1007/s11095-010-0338-9

Kovacsics, D., Raper, J. Transient Expression of Proteins by Hydrodynamic Gene Delivery in Mice. *J. Vis. Exp.*(87), e51481, doi:10.3791/51481 (2014).

Rychahou P.G., Evers B.M. (2010) Hydrodynamic Delivery Protocols. In: Min WP., Ichim T. (eds) RNA Interference. Methods in Molecular Biology (Methods and Protocols), vol 623. Humana Press

Kim MJ, Ahituv N. The hydrodynamic tail vein assay as a tool for the study of liver promoters and enhancers. *Methods Mol Biol.* 2013;1015:279-289. doi:10.1007/978-1-62703-435-7\_18