



GENERAL ITEMS

- ALZET Osmotic Pumps*
- ALZET Rat Intrathecal Catheter* (item #0007740)
- Homeothermic pad
- Sterile drape
- Sterile gauze, cotton swabs
- Hair clippers (#40 blade)
- 70% ethanol
- Betadine solution
- Cotton-tipped applicator
- Glass bead sterilizer
- Ocular lubricant

SURGICAL TOOLS

- Wound Clips (7mm for mice 9mm for rats)*
- Wound Clip Applier, Remover*
- Stereotaxic apparatus
- Scalpel blade (#15), handle (#3)
- Serrated forceps
- Suture (absorbable)
- Suture needle
- Hemostat (straight tip)
- 23 or 25 gauge needle (bent)

* Available from DURECT Corp.
(877-922-5938, alzetcs@durect.com)

The following is a description of the basic surgical procedure for the intrathecal catheterization of the rat via the cisterna magna. Aseptic surgical skills are required to ensure rodent survival. This procedure is to be used only as a guideline for intrathecal cannulation.

Pump Preparation

(refer to the package insert for complete filling instructions)

- Fill the empty ALZET pump with your vehicle or drug solution using a syringe and filling tube according to the procedure listed in the package insert included in your box of pumps.
- Insert the flow moderator into the filled ALZET pump until the cap or flange is flush with the top of the pump.
- Prime the filled pumps in sterile saline at 37°C.
 - Refer to your package insert for the appropriate period of priming. Most pumps require at least an overnight priming period.

Catheter Preparation

- Measure the catheter and cut the distal end (pump attachment portion) to the desired length for intrathecal cannulation.
 - The stylette must not protrude from the catheter. It should be approximately 1-2 mm from the tip.

Anesthesia

(Consult your staff Veterinarian for the appropriate anesthetic dose to use for your study animals)

- Anesthetize the animal using either an inhalable (i.e., Isoflurane) or injectable (i.e., Xylazine® and Ketamine®, or sodium pentobarbital) anesthetic.

Surgical Preparation

- Shave and clean the head and neck.
- Using a sterile swab, disinfect the incision site with 70% ethanol by working outwardly in ever widening circles. With a new sterile swab, apply iodine over the proposed incision site in a similar fashion. Repeat the ethanol and iodine scrub one more time.
- Position the animal in a stereotaxic apparatus ensuring that 1) the head is symmetrical in the device, 2) the pinnas are in a normal, relaxed position, 3) there is no exophthalmus, 4) the head moves freely up and down, but does not move laterally.
- Apply ocular lubricant to the eye.



Surgical Procedure

- Make a midline incision on the skull, extending from a line between the ears to a point approximately 2 cm caudal.
- Find the point on the dorsal head where the bone ends and the muscle begins. Do this by gently grating the blade over the skull, caudally. The bone should be firm, the muscle, soft.
- Cut the muscle where it attaches to the occipital crest about 3 mm lateral on both sides of the muscle midline.
- Peel the muscle from the bone and dura and expose the cisternal membrane at the base of the skull.
- Retract the fascia and tissue from the skull by scraping until exposing 3-4 mm of the cisternal membrane.
 - Large curved forceps may be used to retract the muscle.
- Incise the dura using a bent 25 gauge needle. Exercise considerable care to prevent cutting the dorsal surface of the medulla that lies immediately beneath the membrane. It is common to cut the fascia and not penetrate the dura.
- Copious out dwelling of clear cerebrospinal fluid signals correct incision and intrathecal access.
- Blot the area sparingly to maintain visibility taking care not to remove the fluid from the intrathecal space.
- Flex the animal's neck by lowering the incisor bars on the stereotaxic apparatus. This is done to create maximum space between the cisternal membrane and the spinal cord so that the catheter can be inserted without piercing the spinal cord.
- insert the catheter gently to the desired level using the catheter's natural curve to keep the advancing tip from damaging the spinal cord. As the catheter is inserted, rotate it between the thumb and forefingers to facilitate penetration.
 - Mild twitching of musculature or resistance as the catheter is advanced may be observed. Muscular twitching may signal

stimulation of spinal nerves. The spinal nerves may be damaged if the catheter is further advanced. If resistance is felt stop immediately and pull back on the catheter a small amount and try to advance again.

- The animal's head may be lowered or the thorax may be raised or lengthened to facilitate the advancement of the catheter.
- Once the catheter has been fully advanced, slowly remove the stylette from the catheter.
- Exit the catheter through the musculature at the base of the skull.
- Secure the catheter well around the musculature to prevent pull out.
- Make an incision and create a pocket in the lower mid scapular region to hold the pump.
- Attach the catheter to the flow moderator of the filled ALZET pump.
 - Optional: Prior to connecting the catheter to the ALZET pump, fill the catheter with artificial CSF or test agent using a syringe and needle.
- Insert the filled pump in to the subcutaneous pocket.
- Close the incision with wound clips (7mm for mice, 9mm for rats). Two clips will normally suffice.

Post Operative Analgesia

- An analgesic can be given post-operatively as needed. Analgesic treatment should be provided under the direction of the staff Veterinarian.

Clinical Monitoring and Management

- It is especially important to check the health of the animal the morning after surgery. Animals that reopen the incision site will typically do so after the first night.
- If any adverse effects are seen, the staff Veterinarian will need to be informed immediately for appropriate treatment. Potential adverse effects from this procedure are minimal, but may include the following:
 - Anesthetic-related respiratory depression: Adverse anesthetic effects can be minimized by proper dosing of anesthetic agents and careful monitoring of animals during the anesthetic period.
 - Infection of the subcutaneous pocket: ALZET Osmotic Pumps are provided sterile. Infection can be prevented or minimized if trained surgeons use aseptic surgical techniques and maintain the sterility of products being used. Administration of prophylactic antibiotics may be useful in minimizing the risk of infection, and this should be discussed with the staff Veterinarian.
 - Post-operative pain or discomfort as evidenced by: decreased activity, decreased food and water intake, weight loss, vocalizations, rough hair coat, hunched posture.
- Wound clips must be removed 7-10 days post procedure.