



GENERAL ITEMS

- ALZET Osmotic Pumps*
- ALZET Brain Infusion Kit*
- Loctite 454 Cyanoacrylate*
- Homeothermic pad
- Sterile drape
- Sterile gauze, cotton swabs
- Hair clippers (#40 blade)
- 70 % ethanol
- Betadine scrub
- Glass bead sterilizer
- Ocular lubricant

SURGICAL TOOLS

- AutoClip Wound Clips*
- AutoClip Applier*
- AutoClip Remover*
- Cannula holder 1 or 2*
- Scalpel handle #3
- Scalpel blade #15
- Brown Adson forceps
- Hemostat (straight tip)
- Stereotaxic apparatus
- Cautery (fine tip, low heat)
- Screwdriver
- Screws (stainless steel, 1/8 inch)
- Dremel tool or surgical drill
- Carbide drill burs, fine round tip (e.g. HP-1, or HP-2, REF14823, S White Burs)

* Available from DURECT Corp.
(877-922-5938) alzetc@direct.com

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.

Anesthesia

Anesthesia is required for surgical implantation of ALZET pumps.

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

The use of inhalation anesthetics, such as Isoflurane, is highly recommended. It supplies supplemental oxygen during periods of respiratory depression and provides for rapid anesthetic recovery.

Surgical Preparation

- Apply ocular lubricant to the eye.
- Shave the area centered over the incision site and wash the scalp.

Surgical Procedure

- Place the head of the animal securely and straight in the stereotaxic apparatus with the

tooth bar set at an appropriate level as determined by the same stereotaxic atlas used to establish the cannula coordinates.

- Starting slightly behind the eyes, make a midline, sagittal incision about 2.5 cm long and expose the skull. With the rounded end of a spatula, lightly scrape the exposed skull area and pat it dry. Scraping should remove the periosteal connective tissue adhering to the skull. This enables good adhesion of the cyanoacrylate adhesive, which is later used to secure the cannula. Remove all blood with cotton applicators and stop any remaining bleeding with the low-heat cautery device.
- Identify the bone suture junctions bregma and lambda. With these as reference points, determine and mark the location for cannula placement using the stereotaxic apparatus. Drill a hole through the skull at the marked, stereotaxically correct location, being careful not to penetrate the dura. This hole will receive the cannula.
- Clean and completely dry the skull area where the cannula will be placed by scraping with the scalpel blade or cotton applicators. Additional bleeding should be stopped by cauterizing blood vessels.
- Insert the L-shaped cannula, which is attached by tubing to the ALZET pump, through the skull. To facilitate precise placement of the cannula, the plastic tab located on top of the cannula can be attached to the stereotaxic apparatus. Alternatively, this tab can be used to insert the cannula by hand.

BRAIN INFUSION**KIT 3 (#0008851)**

- Once the cannula has been inserted, gently loosen and remove the cannula holder. The cannula's external arm should lie parallel to the surface of the skull with the tubing extending caudally.
- Drill a second hole partially through the skull and lateral to the cannula. This second hole will be used to receive a small stainless steel screw, which will act as an anchor to secure the cannula.
- Insert the anchor screw while taking care not to go entirely through the cranium. Once the screw has been started into the skull, a turn or two will be sufficient to secure it. The anchor screw should extend approximately 1-2 mm above the skull.
- Completely dry the skull surface and cover the cannula, the entire implantation site, and the anchoring screw with dental cement. The powdered dental cement can be mixed with its acrylic solvent in a dish and applied. Alternatively, the powder can be placed first and the solvent carefully added to it, taking care to limit both to the implantation site. Allow approximately 4 minutes for the cement to set. Note: Many researchers use cyanoacrylate adhesive in place of dental cement. If using cyanoacrylate adhesive, take caution to use a very small amount underneath the base of the cannula to promote proper drying. Using too much adhesive will reduce the adhesive effectiveness. Typically the amount which could cover the end of a toothpick is sufficient.
- Once the cement or cyanoacrylate adhesive is set, prepare a subcutaneous pocket in the midscapular area of the animal's back to receive the ALZET pump. This pocket is created by blunt dissection. A subcutaneous tunnel is also created by blunt-dissection from the scalp incision to the mid-scapular area where the pump is located. The pocket should be large enough to accommodate the pump and permit some pump movement, but not so large as to allow the pump to slip down onto the flank of the animal.
- Insert the ALZET pump (attached to the catheter and brain cannula) into the subcutaneous pocket. The pump should be

placed with the delivery port pointing toward the cannula site. When the pump is properly placed, the catheter should have a generous amount of length to permit free motion of the animal's head and neck. After the cannula is firmly set in place, the plastic tab is easily removed with a heated scalpel, leaving the low-profile cannula in place. A small amount of cement can be applied over the cannula to provide an even smoother surface, conducive to wound healing (not necessary with cyanoacrylate adhesive).

- After the cement has set, close the scalp incision with wound clips or interrupted sutures.

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

- Animals should be monitored daily until the wound clips or sutures are removed then once to twice weekly until completion of the study.
- 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 incision site: ALZET Osmotic Pumps and Brain Infusion Kits 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.