**ALZET® COSMIC PUMP BRAIN INFUSION KIT 2**

**INSTRUCTIONS AND SPECIFICATIONS SHEET**

DURECT Corporation, Cupertino, CA 95014

**I. Technical Description of the ALZET Osmonic Pump Brain Infusion Kit 2**

1. **Brain Infusion Cannula**
   - **Material (tube):** stainless steel
   - **Material (spacer):** polycarbonate
   - **Dimensions (± 0.1 mm):**
     - **A (height, overall):** 11.3 mm
     - **B (height, tab):** 4.5 mm
     - **C (height, tube):** 5.0 mm
     - **D (diameter, flange):** 8.0 mm
     - **E (outside diameter, tubular/idle hole):** 6.3 mm
     - **F (diameter, proximal hole):** 5.0 mm
     - **G (diameter, base):** 5.0 mm
     - **H (diameter, spacer):** 5.0 mm
     - **I (depth, overall):** 2.2 mm

2. **Height Adjustment Spacer**
   - **Material (spacer):** stainless steel
   - **Dimensions (± 0.1 mm):**
     - **D (diameter,tab):** 3.4 mm
     - **H (diameter, spacer):** 5.0 mm

3. **Catheter Tubing**
   - **Material (tube):** polyvinylchloride (medical grade)
   - **Dimensions (± 0.1 mm):**
     - **a (length):** 15 cm (approx.)
     - **b (inside diameter):** 0.69 mm (± 0.08)
     - **c (outside diameter):** 1.14 mm (± 0.08)
     - **d (volume):** 3.74 µl/cm

**II. Checklist for Satisfactory Performance of the ALZET Brain Infusion Model 2**

- Refer to the instructions included with the ALZET osmotic pumps for correct use of these pumps.
- Sterile technique should be used during the filling and handling of osmotic pumps and for the surgical implantation procedure (refer to Sections III and IV of this instruction sheet for correct filling and implantation technique). For more information, refer to the ALZET Brain Infusion Kit Instructions IV.03.
  - Ensure that the device/insertion used is compatible with polyvinylchloride tubing.
- When using the ALZET Brain Infusion Kits, ALZET pumps must be primed, as specified in Section II.04. vs. 7.5 mm of depth.
- The stereotactic coordinates of the target infusion site should be determined before and the cannula must be sterilized. These may vary in your infusion model depending on animal size and stereotactic location.
- Correct placement and patency of the cannula can be verified at the termination of infusion by injecting dye through the cannula.
- Attachment of a catheter to an osmotic pump does not alter its pumping rate.
- The materials in this kit have been exposed to a sterilizing dose of radiation from a Co source.

**III. Instructions for Use of the ALZET Brain Infusion Kit 2**

Preparation of Brain Infusion Assembly

The following steps detail the preparation of the brain infusion assembly (see figure below). This should be done prior to anesthetizing the animal and before filling ALZET osmotic pumps. To prepare the brain infusion assembly, perform the following steps:

1. **Infusion into the cerebrospinal fluid via the cerebral ventricles.**
   - This kit is for use in experimental animals only. It is not to be placed in humans.
   - Direct access to the central nervous system (CNS) via a cannula implanted in the cranium is used in experimental situations where a test compound has effects on the CNS but does not appreciably cross the blood-brain barrier. A test compound can be administered directly to the brain using this technique, allowing its local effects in the brain to be determined independent of its peripheral actions. Administration usually takes two forms:
     - Infusion into the cisternal fluid via the cerebral ventricles.
     - Direct microperfusion of localized regions of solid tissue.

Depending on the nature of the compound administered, intraventricular infusion results in the exposure of a wide range of brain regions to the infusate. In contrast, direct microperfusion usually results in a localized exposure in discrete brain structures. The extent to which different compounds are distributed in brain tissue following local infusion is discussed in the following article:


Note: The stereotaxic coordinates and dimensions listed in these instructions are based on DURECT's experience with brain infusion. They may not be appropriate in your particular application. DURECT recommends that investigators using the ALZET Brain Infusion Kits 2 determine the coordinates and dimensions which provide optimal results in their particular brain infusion model.

Step 2. Without modification, the L-shaped cannula included in this kit will penetrate approximately 5 mm below the surface of the skull. Depending on the animal size, skull thickness, and desired site for infusion, this depth may need to be altered. Spacers are included in this kit to allow you to alter the cannula depth. To do so, slide the desired number of depth adjustment spacers onto the cannula tube and glue these to the wide-base cannula using cyanoacrylate adhesive. The following table can be used to determine the number of spacers needed to achieve the desired depth.

- **Wt:** 3.5 mm
- **L:** 2.0 mm
- **H:** 4.0 mm
- **D:** 2.0 mm

Note: Attachment of more than four spacers to each cannula may not be desirable as this will cause the top of the cannula to protrude greater than 4 mm above the skull. This may make it difficult to close the scalp incision after cannula placement.

A. After attachment of the spacers, verify the length of the remaining cannula tube. Also verify that the tube is straight and that it is at right angles to the bottom of the ellipse. Mount the tube to a support. The tube should protrude from the site by a distance greater than 4 mm from the surface of the cannula and from the interior of the cannula tube.

B. 4.5 cm length of catheter tubing is included in this kit. This tubing can be used to attach the cannula to the flow modulator of the ALZET pump. First, measure the distance between the location at which the cannula will be placed and the site of pump implantation. The cannula must be long enough to reach the pump and should extend beyond the distance between the subcutaneous site of the pump and the location of the cannula, to allow free movement of the animal's head and neck. (For intraventricular infusion, cement the cannula to the cranium 4 mm above the skull. This may make it difficult to close the scalp incision after cannula placement.)

C. Check the catheter tubing by gently pulling on the catheter. Neither end of this assembly should be kinked or easily kinked. If you
IV. Surgical Procedures for Placement of the Cannula and ALZET Osmotic Pump

Note: The stereotaxic coordinates and dimensions listed in these instructions are based on DURECT's experience with brain infusion in rats. They may not be appropriate in your particular application. DURECT recommends that investigators using the ALZET Brain Infusion Kit 2 determine the coordinates and dimensions which provide optimal results in their particular brain infusion model. Information on brain infusion in mice is available from ALZET Technical Services.

Step 1. Anesthetize the mouse or rat (e.g., with an intraperitoneal injection of a solution of sodium pentobarbital, 40-50 mg/kg) and fit the brain infusion assembly.

Step 2. Shave and wash the scalp. Starting slightly behind the eyes, make a midline sagittal incision about 2.5 cm long and expose the skull.

Step 3. Insert the cannula into the skull through the midline hole, 3 mm posterior to the first hole and 4 mm to the left or right of it. Once the screw has been started into the bone, drill a second hole part-way through the skull, 3 mm posterior to the first hole and 4 mm to the left or right of it. This hole should be large enough to accommodate the external arm of the cannula, and the Cannula Holders designed to fit the removable cannula tab of all ALZET Osmotic Pump kits (see above) are available from DURECT Corporation. The cannula holders designed to fit the removable cannula tab of all ALZET Osmotic Pump kits (see above) are available from DURECT Corporation.

Step 4. Completely dry the skull surface. If using dental cement, cover the cannula with dental cement. The powdered dental cement can be mixed with its adhesive to secure both the external portion of the cannula, and the cannula itself to the skull.

Step 5. The stability afforded by this brain cannula, with its wide base and pedicle, may make placement of a stabilizing screw unnecessary. The purpose of a screw would be to provide additional stability, by acting as an anchor to secure the cannula to the skull, and the dental cement that covers and secures the cannula site. If you wish to place a small, stainless steel screw (available from Small Parts, Inc., see Section VII), drill the hole through the skull 3 mm posterior to the first hole and 4 mm to the left or right of it. Once the screw has been started into the bone, drill a second hole with the cannula in place to the midscapular region of the back, taking care not to disturb the integrity of the surgical site.

Step 6. Fill the brain infusion assembly with the solution to be delivered, using the recommended procedure described in the ALZET osmotic pump instructions. If the infusion volume is greater than or equal to the volume specified on the instruction sheet for that model, the pump will not fill properly.

Step 7. Place the flow moderator in the filled osmotic pump. The pump and brain infusion assembly should now be completely filled and free of air bubbles.

Step 8. Insert the cannula into the skull through the midline hole and 4 mm to the left or right of it. Once the screw has been started into the bone, drill a second hole part-way through the skull, 3 mm posterior to the first hole and 4 mm to the left or right of it. The cannula is now ready for implantation.

V. Vertebral Cannula Placement and Patency

Step 1. With a pin vise handle containing a steel bit (e.g., from Small Parts, Inc., see Section VII), drill a hole through the skull at the marked, stereotaxically correct depth. To facilitate precise placement using the stereotaxic coordinates determined in Section III, these as reference points, determine and mark the location for cannula placement.

Step 2. Make the injection using a syringe containing the solution to be delivered, filling the osmotic pump with the solution to be delivered.

Step 3. Insert the cannula into the skull through the midline hole, 3 mm posterior to the first hole and 4 mm to the left or right of it. Once the screw has been started into the bone, drill a second hole part-way through the skull, 3 mm posterior to the first hole and 4 mm to the left or right of it. The cannula is now ready for implantation.

VI. Longer Infusion Periods Using a Single Brain Cannula with Multiple Pumps

Optimal brain infusion results are obtained when a single osmotic pump is used for the full duration of infusion. For delivery periods longer than this, the spent pump, at the end of its pumping duration, must be replaced by a new, fully-loaded and primed pump.

Step 1. Anesthetize the animal. Make a small skin incision in the region of the back, taking care not to disturb the integrity of the brain infusion assembly.

Step 2. Cut the cannula 5-10 mm anterior to the spent pump and remove the pump from the incision.

Step 3. Attach a fresh, fully-loaded pump with flow moderator in place to the freshly cut end of the tubing.

VII. Resources

Stereotaxic Atlases


Stereotaxic Apparatus

Instruments that facilitate the stereotactic placement of ALZET Brain Infusion Kits are available from: Stoelting Co. 620 Woford Lane Wood Dale, IL 60191 Tel: (800) 692-2990 Fax: (630) 860-9775 info@stoelting.com www.stoelting.com David Kopf Instruments 7334 Elmo Street Fullerton, CA 92833 Tel: (1) 714-512-3274 Fax: (1) 714-512-3277 info@kopfindustries.com www.kopfindustries.com Canna holders Canna holders designed to fit the removable cannula tab of all ALZET Brain Infusion Kits are available from DURECT. Canna holder part number 01568B00 (for the cannula holder 1) and part number 01568B02 (for the cannula holder 2) order #0088801.

Pin Vise and Drill Bit

Holes in the skull can be drilled with a steel drill bit (456) and a small pin vise (compatible with steel drill bit #60). These are available from: Amazon.com (formerly Small Parts Inc.) www.amazon.com/Amazon Supply Stainless Steel Machine Screws

Small stainless steel machine screws (size 0-80, 1/8" length with a flatter head) are available from AmazonSupply (see above).

Cyanocrylate Adhesive

Locite 454 (product #008870), a cyanocrylate adhesive gel for affixing the cannula to the skull, is available from DURECT Corporation.

Dental Cement

Dental cement (part number 51458 or 51459) is available from Stoelting Co. (see above).

VIII. Technical Support

Surgical Procedures Available on Video

DURECT offers, free of charge, a video which demonstrates surgical techniques and instructions for all ALZET brain infusion systems. Surgical procedures shown include subcutaneous and intraperitoneal implantations, intrathecal delivery (via the external jugular vein), localized administration in the central nervous system (both intraventricular and intracisternal), gastrointestinal delivery, and other special applications. For a copy of the video, please contact DURECT Technical Support.

ALZET Technical Support

DURECT Corporation
(426) 248-2800
Fax: (426) 248-2809
Cupertino, CA 95014

(800) 692-2990 (U.S. and Canada)
(408) 367-4036 (Outside the U.S.)
(408) 865-1406 (Facsimile)
durect@durect.com
www.durect.com

IX. Warranty

For a period of 12 months from date of shipment, DURECT warrants that the ALZET Brain Infusion Kit 2 ("Product") is free from defects in material and workmanship and conforms to the applicable specifications in this Instructions and Specifications Sheet. The sole and exclusive remedy for any breach of warranty shall be the replacement, at no cost to the customer, of those Units of Product which have been shown to DURECT's reasonable satisfaction to have been defective.

THIS WARRANTY IS VOID ON ANY OF THE FOLLOWING WARRANTIES: EXPRESS OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE, WHICH ARE HEREBY DISCLAIMED. IN NO EVENT SHALL DURECT BE LIABLE FOR ANY SPECIAL, INDIRECT, INCIDENTAL, OR CONSEQUENTIAL DAMAGES. DURECT'S LIABILITY WITH RESPECT TO THE PRODUCT, WHETHER BASED ON CONTRACT, INFRINGEMENT, WARRANTY, NEGLIGENCE, STRICT LIABILITY, OR OTHER LEGAL THEORY, IS LIMITED TO THE REPAIR OR REPLACEMENT COST OF THE PRODUCT, OR THE PRICE PAID BY THE CUSTOMER FOR THE PURCHASING OF THE PRODUCT. SOME JURISDICTIONS DO NOT ALLOW THE EXCLUSION OR LIMITATION OF INCIDENTAL OR CONSEQUENTIAL DAMAGES, SO THE ABOVE LIMITATION OR EXCLUSION MAY NOT APPLY TO YOU.

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