I. Technical Description of the ALZET Brain Infusion Kit 3

Brain infusion assembly is now complete. To prepare the brain infusion assembly, follow the steps below:

1. Selection of the Target Brain Infusion Site
2. Preparation of Brain Infusion Assembly
3. Attaching the Catheter to the ALZET Pump
4. Sterilization and Infection Control
5. Placement of the ALZET Pump into the Animal
6. Monitoring and Termination of Infusion

I.  Technical Description of the ALZET Brain Infusion Kit 3

Infusion into the cerebrospinal fluid via the cerebral ventricles.

Step 1. Determine the correct stereotaxic coordinates for the target infusion site.

Step 2. When using the ALZET Brain Infusion Kits, ALZET pumps must be incubated in sterile saline at 37ºC before implantation (Refer to Section III of this instruction sheet for correct filling and implantation technique).

Step 3. With modification, the L-shaped cannula included in this kit will penetrate approximately 3 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 them to the wide cannula base using cyanoacrylate adhesive. The following table can be used to determine the number of spacers needed to achieve the desired depth. Note that the thickness of the spacers is 0.5 mm.

<table>
<thead>
<tr>
<th>Number of Spacers</th>
<th>Depth of Cannula Remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.0 mm</td>
</tr>
<tr>
<td>1</td>
<td>2.5 mm</td>
</tr>
<tr>
<td>2</td>
<td>2.0 mm</td>
</tr>
<tr>
<td>3</td>
<td>1.5 mm</td>
</tr>
<tr>
<td>4</td>
<td>1.0 mm</td>
</tr>
</tbody>
</table>

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

Step 4. After attachment of the spacers, verify the length of the remaining cannula tube. Also verify that the tube is straight and that it is free of any kinks in the region of the subcutaneous incision. Should the cannula become contaminated during this procedure, soak it in an aqueous solution of 70% ethanol for several minutes. Before implantation, allow the ethanol to evaporate from the surface of the cannula and from the interior cannula lumen. The catheter which connects the cannula to the pump should be 25% longer than 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.

Step 5. Cut the catheter tubing to the length determined in Step 4. Attach one end of the tubing to the flow moderator 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 catheter which connects the cannula to the pump should be 25% longer than the distance between these two locations. Using the appropriate tubing, cut the catheter tubing to the desired length. Insert the catheter tubing into the cannula and flow moderator, and glue it in place using cyanoacrylate adhesive. The brain infusion assembly is now complete.

* The ALZET Brain Infusion Kit 3 has been designed specifically to work with ALZET osmotic pumps. For further information on ALZET pumps, please contact ALZET Technical Support at (800) 692-2990 or (408) 367-4036.

For animal use only.

II. Checklist for Satisfactory Performance of the ALZET Brain Infusion Kit 3

1. Checklist for Satisfactory Performance of the ALZET Brain Infusion Kit 3

- 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)
- Ensure that the vehicle/solvent used is compatible with polyvinylpyrrolidone.
- When using the ALZET Brain Infusion Kits, ALZET pumps must be incubated in sterile saline at 37ºC before implantation.
- The stereotaxic coordinates of the target infusion site should be determined and the desired cannula length calculated prior to surgery. These may vary in your infusion model depending on animal size and stereotaxic 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 3

Preparation of Brain Infusion Assembly

- For animal use only.
Implantation.

Step 1. The pump and brain infusion assembly are now ready for implantation. It also minimizes the risk of clotting within the cannula or recommended in the instructions for the pump model being used.

Step 2. Confirm cannula placement.

Step 3. Cut the catheter and slowly inject a dye (e.g., Evans Blue) through the catheter toward the cannula. Expose the tip of the cannula on the floor of the brain.

Step 4. The pump and brain infusion assembly should now be completely filled and free of air bubbles.

IV. Surgical Procedures for Placement of the Cannula and ALZET Osmotic Pump

Note: The stereotactic coordinates and dimensions listed in these instructions are based on DURECT’S experience with brain infusion in rats. They may not be applicable in your particular application.

DURECT recommends that investigators using the ALZET Brain Infusion System 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 pentobarbital, 40-50 mg/kg) and fit the animal with a stereotaxic apparatus (e.g., from Stoelting, see Section VII).

Step 2. (Shave and wash the scalp). Stirring slightly behind the eyes, make a midline sagittal incision and expose the skull. With the rounded end of a scalpel, quickly expose the exposed skull area and pat. Scraping should remove the periosteal connective tissue which adheres to the skull, permit easy adhesion of the dental cement which is used later to secure the cannula.

Step 3. Prepare a subcutaneous pocket in the midlokatary area of the head of the animal for the osmotic pump. This pocket is created by using a personal scalpel or a heated scalpel (such as using a heated scalpel) and the cannula placed by hand. The external arm of the cannula should now lie parallel to the surface of the skull.

Step 4. Identify the bone suture junctions, bregma and lambda. With these as reference points, determine and mark the location for cannula placement using the stereotaxic coordinates described in Section III.

Step 5. With a pin vise handle containing a steel bit (e.g., from Plastics One, see Section VII), drill a hole through the skull at the stereotaxically correct location. This hole will receive the cannula.

Step 6. Stability afforded by this brain cannula, with its wide base and flat profile, may make placement of a visualization-stabilizing cement recommended. The purpose of a screw would be to provide an additional stability, by acting as an anchor to secure both the external portion of the cannula, and the dental cement that covers and secures the cannula site. If you wish to place a small, stainless steel screw, drill a second hole part-way through the bone located anterior to the first hole and 4 mm to the left or right of it. Once the screw has been started into the skull, a turn or two is sufficient to secure it. The screw should be inserted approximately 2 mm above the skull.

Step 7. Insert the cannula into the subcutaneous pocket, leading the cannula out of the site for cannula placement. The cannula should be placed with the delivery port pointing toward the cannula site. When the cannula is properly placed, the catheter should have a generous amount of slack to permit free motion of the animal’s head and neck.

Step 8. If using dental cement, cover the cannula, the entire cannula site, and the anchoring screws with dental cement. The powdered dental cement should be mixed with its acrylic solvent in a dish. Alternatively, the powder can be placed first over the cannula and the solvent carefully applied to the cannula. Care should be taken not to drip any cement or solvent into the animal’s eyes.

Note: Adhesion of the cannula to the skull can be improved if canine is taken to clean tissue from the skull surface around the cannula site, and the site is dry.

Step 9. Allow the cement to harden for four minutes and remove the placement tab from the top of the cannula if it has not already been removed. The scalp wound can now be closed with suture.

Step 10. Remove the animal from the stereotaxic apparatus and place it back in its cage. The animal requires no restraint or handling during the delivery period.

V. 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 that of an osmotic 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 midlokatary region of the spine, taking care not to disturb the integrity of the brain infusion assembly.

Step 2. Clamp the catheter using a non-traumatic haemostat. Cut the catheter 5-10 mm anterior to the apex pump and remove the clamp from the infusion.

Step 3. Clamp the cannula using a non-traumatic haemostat. Remove the catheter, 5-10 mm anterior to the cannula site, and replace the cannula using a non-traumatic haemostat.

VII. Resources

Stereotactic Atlases

Stereotactic data for placement of catheters and cannulae is available in:


Stereotaxic Apparatus

Instruments that facilitate the stereotactic placement of ALZET Brain Infusion Kits are available from:

- Stoelting Co.
  620 Wheatland Lane
  Wood Dale, IL 60191
  Tel.: (800) 860-9775
  Fax: (630) 860-9775
  Email: info@stoelting.com
- Sjogren, Inc.
  9319 S. 24th St.
  Omaha, NE 68106
  Tel.: (818) 352-3074
  (877) 352-3275
  Fax: (818) 352-3135
  Email: sales@sfctools.com

Cannula Holders

Cannula holders designed to fit the removable cannula tab of all ALZET Brain Infusion Kits are available from DURECT Technical Support 0008860.

Pin Vise and Drill Bit

Holes in the skull can be drilled with a steel drill bit (0.05) and a small pin vise with steel drill bit 0.0456). These are available from:

- Plastic One Inc.
  6591 Minterman Rd
  Roseville, CA 95661
  Tel.: (540) 772-1166
  Email: info@plasticsone.com

Dermal, Stainless Steel Machine Screws

Small stainless steel machine screws (size #0-40, 1/8" in length with a hillster head) are available from Plastic One (see above).

Cyanoacrylate Adhesive

DURECT sells Locite 454 (#item 0020670), a cyanoacrylate gel for affixing the cannula to the skull.

Surgical Procedures Available on Video

DURECT offers, free of charge, a video which demonstrates surgical techniques and special applications for ALZET osmotic pumps. Surgical procedures include intracerebral and intrathecal, gastrointestinal delivery, and other specialized applications. To receive a copy of the video, please contact:

ALZET Technical Support

DURECT Corporation

2042 Bubo Road

Cupertino, CA 95014

Tel.: (800) 692-2395

Fax: (408) 407-4007

(US and Canada)

Tel.: (805) 865-1406

(Outside the US and Canada)

Email: alzet@durect.com

www.alzet.com (web site)

email: info@plastics1.com

www.kopfinstruments.com

email: sales@kopfinstruments.net

www.ceptal.com

email: info@ceptal.com

www.devotec.com

email: sales@devotec.com

www.stoelting.com

email: info@stoelting.com

www.sfctools.com

email: sales@sfctools.com

www.franklinchron.com

email: franklinchron.com

www.tes.net

email: sales@tes.net

www.crescendo.com

email: sales@crescendo.com

www.plasticsone.com

email: info@plasticsone.com

www.alzet.com

email: info@alzet.com

www.dancock.com

email: info@dancock.com

www.coperon.com

email: info@kopfinstruments.com

www.kopfinstruments.com

email: sales@kopfinstruments.net

www.devotec.com

email: sales@devotec.com

www.stoelting.com

email: info@stoelting.com

www.franklinchron.com

email: franklinchron.com

www.tes.net

email: sales@tes.net

www.crescendo.com

email: sales@crescendo.com

www.plasticsone.com

email: info@plasticsone.com

www.alzet.com

email: info@alzet.com

www.dancock.com

email: info@dancock.com