**I. Brain Infusion Cannula**

<table>
<thead>
<tr>
<th>Description</th>
<th>Material</th>
<th>Dimensions (± 0.1 mm)</th>
<th>Inside Diameter (± 0.08)</th>
<th>Outside Diameter (± 0.08)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Length</strong></td>
<td>stainless steel</td>
<td>0.5 mm</td>
<td>0.71 mm</td>
<td>1.14 mm (± 0.08)</td>
</tr>
<tr>
<td><strong>Height, overall</strong></td>
<td>polyvinylchloride</td>
<td>17.1 mm</td>
<td>5.9 mm</td>
<td>6.2 mm</td>
</tr>
<tr>
<td><strong>Depth, base</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>A</strong></td>
<td></td>
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<tr>
<td><strong>B</strong></td>
<td></td>
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<td></td>
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<tr>
<td><strong>C</strong></td>
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<td></td>
</tr>
<tr>
<td><strong>D</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

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

1. Refer to the instructions included with the ALZET Osmotic Pumps for correct use of these pumps.
2. The ALZET pump should be primed using saline at 37°C before implantation.
3. The termination of infusion by injecting dye through the cannula.
4. Correct placement and patency of the cannula can be verified at the termination of infusion by injecting dye through the cannula.

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

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. Select the appropriate cannula length for your application. The ALZET Brain Infusion Kit 2 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 alzet@durect.com.

2. After attachment of the spacers, verify the length of the cannula and adjust as necessary to achieve the depth desired.

3. The ALZET Brain Infusion Kit 2 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 alzet@durect.com.
about the security of this assembly, cement the catheter to the cannula and flow-modified cannula with cyanoacrylate adhesive. The brain infusion assembly is now complete.

Step 6. Fill the brain infusion assembly with the solution to be delivered. The contents of the syringe containing the solution to be delivered to the remaining catheter tubing and connect the tubing to the free end of the flow modulator.

Step 7. Fill the cosmetic pump with the solution to be delivered, using the recommended procedure described in the ALZET Osmotic Pump Instruction Sheets. Assurant that the fill volume is greater than or equal to the volume specified on the instruction sheet for that model.

Step 8. Place the flow modulator in the filled cosmetic pump. The pump and brain infusion assembly should now be completely filled and free of air bubbles.

Step 3. Incubate the filled brain infusion assembly with attached cosmetic pump in sterile saline (0.9%) at 37°C for the time period recommended in the surgical procedures shown or in the model being used. This step is mandatory when ALZET Osmotic Pumps are used with a catheter, as it ensures that the pump is pumping continuously prior to implantation. It also minimizes the risk of clotting within the cannula or occlusion by tissue during delivery of the test agent.

Step 10. The pump and brain infusion assembly are now ready for implantation.

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 applicable in all species. For additional information, DURECT recommends that investigators using the ALZET Brain Infusion System 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 Support.

Step 1. Anesthetize the mouse or rat (e.g., with an intraperitoneal injection of a solution of xylazine-pentobarbital, 40-50 mg/kg) and place the animal in a stereotaxic apparatus (e.g., from Stoelting Co., see Section VI).

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. With a hemostat, grasp the skin edges of the scalp, lift open the skin, and pull it d. Scraping should remove the peristomal connective tissue which adheres to the skull. Allow the scalp to dry and cover the area with the alcohol.

Step 3. Prepare a subcutaneous pocket in the midcervical area of the back of the animal for the cosmetic pump. This pocket is created by using a hemostat to make a small subcutaneous incision in the area above the midcervical area and then opening and closing the hemostat to form a pocket. 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 to the flank of the animal.

Step 4. Identify the bone suture junctions, bregma and lambda. With a damaged stereotaxic instrument (e.g., from Stoelting Co., see Section VII), drill a hole through the skull at the marked, stereotaxic correct location. This hole will receive the cannula.

Step 5. Position the flow moderator arm of a stereotaxic instrument (e.g., from Stoelting Co.) in the midline hole, insert the brain cannula through the skull, and pass the catheter down the cannula. Using the midline hole, insert the brain cannula through the skull. Using the midline hole, insert the brain cannula through the skull. To do this, attach a correct depth. To facilitate precise placement of the cannula, the tab on the top of the cannula can be attached to the electrode holder arm of a stereotaxic instrument (e.g., from Stoelting Co.). Alternatively, the tab can be attached to the cannula (e.g., using dental acrylic adhesive) and the cannula placed by hand. The external arm of the cannula can now be tied to the surface of the skull.

Step 6. Completely dry the skull surface. If using dental cement, cover the cannula, the entire cannulation site, and the anchoring screws with dental cement. The powdered dental cement can be mixed with its acrylic solvent in a dish. Alternatively, the powder can be placed first over the cannula, the solvent carefully added to it. 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 care is taken not to drip any cement or solvent into the animal's eyes.

Step 7. If using cyanoacrylate adhesive, apply a thin layer to the base of the cannula. Using the midline hole, insert the brain cannula through the skull. To do this, attach a correct depth. To facilitate precise placement of the cannula, the tab on the top of the cannula can be attached to the electrode holder arm of a stereotaxic instrument (e.g., from Stoelting Co.). Alternatively, the tab can be attached to the cannula (e.g., using dental acrylic adhesive) and the cannula placed by hand. The external arm of the cannula can now be tied to the surface of the skull.

Step 8. Cut the catheter and slowly inject a dye (e.g., Evans Blue) through the cannula and examine the dye stains to confirm its placement. Alternatively, after the catheter has been removed, the brain can be fixed, frozen, and sectioned to confirm cannula placement.

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 the life span of the animal, at the end of 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 neck region of the back, taking care not to disturb the integrity of the brain infusion assembly.

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

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

VII. Resources

Stereotaxic Atlases

Stereotaxic 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 Wheat Lane Wood Dale, IL 60191 Tel: (888) 201-9090 Fax: (630) 960-9775 email: info@stoelting.com www.stoeltingco.com

David Kopf Instruments 7224 Elmo Street Santa monica, CA 90405 Tel.: (877) 552-3275 Fax: (918) 352-3139 email: sales@kopfinstruments.net www.kopfinstruments.com

Cannula Holders

Cannula holders designed to fit the removable cannula tab of all ALZET Brain Infusion Kits are available from DURECT. Cannula Holder 1 (order #000889) and Cannula Holder 2 (order #000881).

In Vivo Inc.

Stainless Steel Screws

Small stainless steel screws (size 40-80, 1/8" in length with a filet) are available from Plastics One Inc. (see above).

Cyanoacrylate Adhesive

Loctite 454 (order #000867), a cyanoacrylate adhesive gel for affixing the cannula to the skull, is available from DURECT. A small amount of the cyanoacrylate can be injected directly into the skull, and the cannula then affixed to the skull using the cyanoacrylate. The bone suture should be tied approximately 2 mm above the skull.

Stereotaxic Apparatus

Stereotaxic Apparatus

DURECT Technical Support

www.invivo1.com

DURECT Corporation 620 Wheat Lane Suite 302B scalable Email: sales@durect.com www.durect.com

6591 Merriman Rd Rancho, CA 91406 Tel.: (540) 772-1186 Email: info@invivo1.com

www.kopfinstruments.com

www.stoeltingco.com

www.invivo1.com

DURECT Corporation 620 Wheat Lane Wood Dale, IL 60191 Tel: (888) 201-9090 Fax: (630) 960-9775 email: info@stoelting.com www.stoeltingco.com

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www.kopfinstruments.com

www.stoeltingco.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 manufacture and conforms to the applicable specifications in this Instructions and Specifications Sheet. The sole and exclusive remedy for any breach of warranty shall be, at DURECT's option, replacement of the Product without charge, or refund of the purchase price of the Product. DURECT disclaims any and all liabilities, expressed, implied or otherwise for loss of income, business, profit, or any special, indirect, or consequential loss. DURECT shall not be liable for any indirect, incidental, special, punitive, or consequential damages, whether caused by negligence or otherwise, which results from the use of, or inability to use, the Product. THIS WARRANTY IS VOID IN THE EVENT OF ANY ALTERATION OF THE PRODUCT, OR IN THE EVENT OF THE CUSTOMER FAILURE TO FOLLOW THE INSTRUCTIONS AND SPECIFICATIONS SHEET ACCURATELY, OR IN THE EVENT OF ANY OTHER CAUSE WHICH IS DUE TO THE CUSTOMER. IN NO EVENT SHALL DURECT'S LIABILITY EXCEED THE PRICE PAID BY THE CUSTOMER FOR THE PURCHASE OF THE PRODUCT GIVING RISE TO SUCH LIABILITY.