Du/RECT Corporation offers a series of miniature implantable pumps, ALZET osmotic pumps, for use in laboratory animals. ALZET Brain Infusion Kit 1 is for use with ALZET pumps for local delivery of test solutions to the central nervous system (CNS). The ALZET Brain Infusion Kit 1 is used for intracerebroventricular infusion, or for targeted delivery to specific solid tissue structures within the brain. When used correctly, ALZET pumps and the ALZET Brain Infusion Kit 1 permit continuous delivery of compounds for extended periods of time without the need for external connections or frequent handling of the animal.

This kit is for use in experimental animals only. It is not to be placed in the subcutaneous or intraperitoneal space. This kit is not to be used in humans. Direct access to the central nervous system (CNS) via a cannula implanted in the cranial dura mater is not recommended. There are reports of brain damage if uncontrolled cannula placement results in the exposure of a wide range of brain regions.

Depending on the nature of the compound administered, intravenous, intracerebral, intraventricular, or intrasubcutaneous infusion in Sprague-Dawley rats weighing approximately 250 g. However, an infusion site of 4-5 mm is appropriate for intraventricular infusion in Sprague-Dawley rats weighing approximately 250 g. Optimal results in your intraventricular infusion model may be achieved at different depths. Two stereotaxic atlases are listed in the resources section at the end of this instruction sheet.

I. Technical Description of the ALZET Brain Infusion Kit 1

Du/RECT Corporation, Cupertino, CA 95014

I. Technical Description of the ALZET Brain Infusion Kit 1

ALZET® OSMOTIC PUMP

Instructions and Specifications Sheet

LOT NO:

Here is a table summarizing the dimensions of the Brain Infusion Cannula:

<table>
<thead>
<tr>
<th>Dimensions (a, µm)</th>
<th>Length</th>
<th>Inside diameter</th>
<th>Outside diameter</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (height, overall)</td>
<td>12.5 mm</td>
<td>6.8 mm</td>
<td>5.0 mm</td>
<td>0.81 mm</td>
</tr>
<tr>
<td>B (length, overall)</td>
<td>3.4 mm</td>
<td>0.36 mm</td>
<td>0.00 mm</td>
<td>0.032 mm</td>
</tr>
<tr>
<td>H (height, spacer)</td>
<td>2.4 mm</td>
<td>0.5 mm</td>
<td>0.0 mm</td>
<td>0.000 mm</td>
</tr>
</tbody>
</table>

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

1. Refer to the instructions included with ALZET osmotic pumps for correct use of these pumps.
2. Sterile technique should be used during the filling and handling of osmotic pumps and the surgical implantation procedure (refer to Section III of this instruction sheet for correct filling and implantation techniques).
3. For use with a catheter, ALZET pumps must be imbued in sterile saline solution (see Chapter 2 for further information on ALZET pumps, please contact the Technical Services Desk at (603) 450-2000 or (408) 367-4026.

Step 1. Determine the correct stereotaxic coordinates for the target cannula site, and calculate the desired cannula length. For example, a cannula length of approximately 4.5 mm is appropriate for intraventricular infusion in Sprague-Dawley rats weighing approximately 250 g. However, optimal results in your intraventricular infusion model may be achieved at different depths. Two stereotaxic atlases are listed in the resources section at the end of this instruction sheet.

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's size and weight, and desired site for infusing, 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 spacers into the cannula length to project greater than 4 mm above the skull. This may make it difficult to close the incision after cannula placement.

Step 3. After attachment of the spacers, verify the length of the remaining cannula tube. Also verify that the tube is straight and that it is an angle to the skull and anti-parallel to the midscapular region on the back. This requires about 10 cm of tubing in place before implantation. The cannula may not be appropriate in your particular application. DURECT recommends that investigators using the ALZET Brain Infusion Kit 1 determine the coordinates and dimensions which provide optimal results in their particular brain infusion model.

Note: stereotaxic coordinates and dimensions listed in these instructions are based on Du/RECT's experience with brain infusion. The coordinates listed here are not intended to be used for surgical implantation techniques.

III. Instructions for Use of the ALZET Brain Infusion Kit 1

Preparation of Brain Infusion Assembly

The following steps detail the preparation of the brain infusion assembly. These steps can be done prior to anesthetizing the animal and before filling ALZET osmotic pumps. To prepare the brain infusion assembly, perform the following steps:

1. Attach one end of the tubing to the cannula and the other end to the flow moderator of the ALZET pump. Tubing should be 25% longer than 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 the subcutaneous site of the pump and the location of the cannula. Place the attached tubing into the cannula and neck. (For intraventricular infusion, cement the cannula to the cranium 3 mm posterior to bregma and place the pump in the midscapular region on the back. This requires about 10 cm of tubing in a rat weighing 250 g).

Step 4. Cut the catheter tubing to the length determined in Step 4. Attach one end of the tubing to the cannula and the other end to the ALZET pump flow modulator. Check the attachment by gently pulling on the catheter. Failure of this assembly should be loose or easily dislodged. If you are concerned about the security of this assembly, contact the cannula to the cranium and flow modulator with cyanoacrylate adhesive. The brain infusion assembly is now complete.

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 incision after cannula placement.

Contact: Sendelbeck SL and Urquhart J. Spatial distribution of dopamine, 2990 or (408) 367-4036.

Address: 2990 University Ave, Suite 700, Santa Clara, CA 95054

Phone: (408) 855-2990 or (408) 367-4036.

Fax: (408) 855-2990.

**ALZET® Brain Infusion Kit 1**

Prepared by: Durect Corporation

Printed in U.S.A.

April 2006

©2006 DURECT Corporation

DURECT Corp.
Step 6. Fill the brain infusion assembly with the solution to be delivered. To do this, attach a syringe containing the solution to be delivered to the remaining catheter tubing and connect the tubing to the free end of the flow moderator. When the pump is used for the full duration of infusion, the solution is replaced by a new, fully-loaded and primed pump. However, when the pump is used for the full duration of infusion, the solution may not be appropriate in your particular application. DURECT recommends that investigators using the ALZET Brain Infusion Kit 1 review the stereotaxic coordinates and dimensions which provide optimal results in their particular brain infusion model. Information on brain infusion in mice is available at DURECT Technical Services.

Step 1. Anesthetize the rat (i.e., with a intraperitoneal injection of a solution of sodium pentobarbital, 40 - 50 mg/kg) and fit the rat in a stereotaxic apparatus (e.g., from Stoelting, see Section VII).

Step 2. Shave and wash the scalp. Starting slightly to the eyes, make a midline sagittal incision about 2.5 cm long and expose the skull. Carefully strip the exposed bone of a scalp, lightly scrape the exposed bone with a trephine and put it dry. Scoping should remove the pericranial connective tissue which adheres to the skull, permitting good adhesion of the dental cement which is used later to secure the cannula.

Step 3. Prepare a subcutaneous pocket in the midcranial area of the skull for the cannula. This pocket is created by a hemostat to make a short subcutaneous tunnel from the scalp incision to the cannula implantation site. As the pocket is formed the pocket should be large enough to accommodate the pump and permit some pump movement, but small enough to allow the pump to slip down to the floor of the animal.

Step 4. Identify the bone suture (junctions, bregma and lambda). With appropriate reference points, determine and mark the location for cannula placement using the stereotaxic coordinates determined in Section III, stereotaxically correct location. This hole will receive the cannula.

Step 5. Drill a second hole in the skull, 3 mm posterior to the first hole and 1 mm to the right or left of it. This second hole will be used to place the delivery port pointing toward the cannula site. Because the cannula is made of stainless steel, it is drilled part way through the skull. The small screw acts as a pin to secure both the external portion of the cannula and the dental cement which will cover and secure the entire implantation site.

Step 6. Insert the small anchor screw while taking care not to go through the skull. The anchor screw should be inserted into the brain infusion assembly. The cannula 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 slack to permit free motion of the animal’s head and neck.

Step 7. Insert the small anchor screw while taking care not to go through the skull. The cannula is made of stainless steel, it is drilled part way through the skull. The small screw acts as a pin to secure both the external portion of the cannula and the dental cement which will cover and secure the entire implantation site.

Step 8. Remove the jaw and roof of the mouth of the animal and examine the dye stains to confirm its placement. Alternatively, after the cannula is removed, the brain can be fixed, frozen, and sectioned to confirm cannula placement.

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

Optimal brain infusion results are obtained when a single cannula 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 removed and a new fully-loaded and primed pump replaced. To do this, attach a syringe containing the solution to be delivered to the remaining catheter tubing and connect the tubing to the free end of the flow moderator. When the pump is used for the full duration of infusion, the solution is replaced by a new, fully-loaded and primed pump.

Step 1. Anesthetize the rat. Make a small skin incision in the midcranial region of the back, taking care not to distort the integrity of the brain infusion site.

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

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

VIII. Resources

Stereotaxic Atlases

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


Stereotaxic Apparatus and Cannula Holder

Heavy duty electrodes holder fits the removable cannula lab and facilitates stereotaxic placement. These are available from:

Stoelting Co.

(Cannula holder part # 51613)

620 West Lake

Wood Dale, IL 60191

Tel.: (630) 860-9700

Fax: (630) 860-6711

email: physiology@stoeltingco.com

www.stoeltingco.com

Durect-Kopf Instruments

(Cannula holder part # 1776-APA or 1776-P1)

11111 West Street

Tujunga, CA 91042

Tel.: (818) 352-3274

Fax: (818) 352-3273

email: smarts@smparts.com

Stainless Steel Machine Screws

Small stainless steel machine screws (size 40-40, 1/8’’ length with a #00 head, part number MX-0080-02FL) are available from Small Parts, Inc. (see above).

Pin Vice and Drill Bit

Holes in the skull can be drilled with a steel drill (size 56, part number 561000-100) and a small pin vice (part number 561000-103). These are available from:

Small Parts, Inc.

13980 N.W. 58th Court

P.O. Box 4650

Miami Lakes, FL 33014-0650

Tel.: (800) 220-4424

Fax: (800) 220-4423

email: smarts@smparts.com

www.smparts.com

Stainless Steel Machine Screws

Small stainless steel machine screws (size 40-40, 1/8’’ length with a #00 head, part number MX-0080-02FL) are available from Small Parts, Inc. (see above).

Cylindrical Adhesive

Durect sells Locitite 454 (product # 009870, a cyanoacrylate adhesive gel, for affixing the catheter to the skull.

Dental cement

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

IX. Warranty

For a period of 12 months from date of shipment, DURECT warrants that the ALZET Brain Infusion Kit 1 (‘‘Product’’) is free from defects in materials 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 of the Product at no cost to the consumer, of those products which have been shown to DURECT’s reasonable satisfaction to have been defective.

This warranty is in lieu of all other warranties, express or implied, and specifically excludes any and all warranties of merchantability and fitness for a particular purpose, which are hereby excluded. In no event shall DURECT be liable for any indirect, incidental, special, or consequential damages, including but not limited to lost profits, even if DURECT has been advised in advance of the possibility of such damages.

This warranty applies to products sold to U.S. and Canadian consumers.

This warranty does not apply to products sold to non-U.S. consumers or non-Canadian consumers.

This warranty is null and void if any defects have been shown to DURECT’s reasonable satisfaction to have been caused by: