ALZET® BRAIN INFUSION KIT 3 INSTRUCTIONS AND SPECIFICATIONS SHEET

DURECT Corporation offers a series of miniature implantable pumps, ALZET Osmotic Pumps, for use in laboratory animals. The ALZET Brain Infusion Kit 3 is for use with ALZET pumps for local delivery of test solutions to the central nervous system (CNS). The ALZET Brain Infusion Kit 3 can be used for intracerebroventricular infusion, or for targeted delivery to specific solid tissue structures within the brain.

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


Local infusion can be utilized in brain regions which may be bypassed by systemic delivery (such as the brainstem and deep brain structures). It may be the only method available to deliver test compounds to certain brain regions. It can be used to determine the effects of drug delivery on a regional basis. Direct access to the central nervous system (CNS) via a cannula implanted in the cranium is useful 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:

1.  Infusion into the cerebrospinal fluid via the cerebral ventricles.
2.  Direct microperfusion of localized regions of solid brain tissue.

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


As described in Step 1, the brain infusion cannula is implanted in the skull of the animal. As the cannula becomes contaminated during this procedure, soak it in an aqueous solution of 70% ethanol for several minutes. Before implantation, ensure that the cannula is free of any foreign debris, and that the top of the cannula is free of any debris.

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 sterilized dose of radiation from a Co60 source.

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I. Technical Description of the ALZET Brain Infusion Kit 3

1. Brain Infusion Cannula

A. Material (tube) (stainless steel)
   Polycarbonate

Dimensions (± 0.1 mm)

- A (height, overall)   9.3 mm
- B (length, overall)  10.0 mm
- C (height, tube)   3.0 mm
- D (diameter, tab)  0.4 mm
- E (outside diameter, distal inlet)  0.31 mm
- F (outside diameter, proximal inlet)  0.71 mm
- G (diameter, base)   5.9 mm
- H (outside diameter, tube)  0.31 mm
- J (thickness, spacer)   0.5 mm

Volume inside tube   0.23 μl
Volume remaining in tube (attached)   10.7 μl
Volume remaining in tube (not attached)   3.7 μl

2. Height Adjustment Spacer

A. Material (polycarbonate)

Dimensions (± 0.1 mm)

- A (height, spacer)  5.9 mm
- B (diameter, spacer)   0.5 mm

Number of Spacers

- 1.0 mm
- 1.5 mm
- 2.0 mm
- 2.5 mm
- 3.0 mm

Attached  1
Tube Remaining  10

3. Catheter Tubing

A. Material (polycarbonate)

Dimensions

- Inside diameter   0.59 mm ± 0.08
- Outside diameter  1.14 mm ± 0.08
- Length   3.74 μl/cm

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

1. Refer to the instructions included with the 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 cannula length processed prior to surgery. (Refer to Section III and IV of this instruction sheet for correct filling and implantation techniques.)
3. Ensure that the vehicle/solvent used is compatible with polyvinylidene tubing.
4. When using the ALZET Brain Infusion Kits, ALZET pumps must be incubated in sterile saline solution before implantation. (Refer to Section III of this instruction sheet for complete instructions.)
5. 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.
6. Correct placement and patency of the cannula can be verified at the termination of infusion by injecting dye through the cannula.
7. Attachment of a catheter to an osmotic pump does not alter its pumping rate.
8. The materials in this kit have been exposed to a sterilized dose of radiation from a Co60 source.

III. Instructions for Use of the ALZET Brain Infusion Kit 3

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 the ALZET Osmotic Pump. To prepare the brain infusion assembly,

1. The ALZET Brain Infusion Kit 3 has been designed especially to work with ALZET Osmotic Pumps. For further information on ALZET pumps, please contact ALZET Technical Support at (800) 862-2950 or alzet@alzet.com.

Step 1. Determine the correct stereotaxic coordinates for the target in vivo site, and calculate the desired cannula length. For example, a cannula length of approximately 2-3 mm is appropriate for infusion into the third or lateral ventricle in most species. However, optimal results in your intraventricular infusion model may be achieved at different lengths. Contact DURECT for assistance in selecting the resources section at the end of this instruction sheet.

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 Kit 3 consult the user manuals and references which provide optimal results in their particular brain infusion model.

Step 2. Without modification, the L-shaped cannula included in this kit will fit the pump assembly. It is important that the cannula length is determined and the desired cannula length calculated prior to surgery. Depending on the animal size, skull thickness, and desired site for infusion, the desired length can be altered. These spacers are included in this kit to allow you to vary the cannula length. To do so, slide the desired number of depth adjustment spacers onto the cannula tube and glue these 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>Attachment/Tube 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 place the scap 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 at right angles to the bottom of the elbow stop and spacers. Should the cannula tube become bent or kinked during this procedure, soak it in an aqueous solution of 70% ethanol for several minutes. Before implantation, ensure that the cannula is free of any foreign debris, and that the top of the cannula is free of any debris.

Step 4. A 15 cm length of catheter tubing is included in this kit. This tubing is used for the local delivery by moderate injection of the ALZET pump. First, measure the distance between the location at which the cannula will be placed and the site of pump implantation. Then, subtract 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 cannula and the other end to the ALZET pump flow modulator.

Note: Attaching catheter to the pump. 200 μl & 2 ml Models:

Remove the translucent cap from the end of the flowmodulator, revealing a short stainless steel tube protruding from the white flange. 110 μl Models: Remove and discard the plastic white flange using scissors or pliers. In doing so, be careful not to bend or crush the stainless steel tube. These pump models are designed to be as space efficient as possible for use in smaller animals, so they were designed without the translucent cap on the flowmodulator. Check the attachment by gently pulling on the catheter. If both of these statements are true, this catheter should be loose or easily distorted. If you are concerned about the security of this assembly, contact the cannula and flowmodulator manufacturer to arrange replacement with cyanoacrylate adhesive. The brain infusion assembly is now complete.
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 appropriate in your particular application.

DURECT recommends that investigators using the ALZET Brain Infusion Kit 3 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 xilazine pentobarbital, 40-50 mg/kg) and fit the animal into a stereotaxic apparatus (e.g., from Stereotax, see Section VII).

Step 2. Shave and wash the scalp. Slash slightly behind the eyes, make a midline sagittal incision and expose the skull. With the round and oval bur, remove the exposed skull and scalp. Scrub the bur for 30 seconds and reinsert. Remove the bur for 30 seconds and reinsert.

Step 3. Prepare a subcutaneous pocket in the midback area of the animal for the osmotic pump. This pocket is created by using a heated scalpel to remove (e.g., using a heated scalpel) and the cannula placed by hand.

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 stereotactic coordinates described in Section VIII.

Step 5. With a pin vise handle containing a steel bit (e.g., from Plastics One Inc.) and a drill bit, drill a hole through the skull and into the selected stereotaxic correct location. This hole will receive the cannula.

Step 6. Fill the osmotic pump with the solution to be delivered, using the flow moderator.

To do this, attach a syringe containing the solution to be delivered to the pump and fill the pump to the volume specified on the instruction sheet for that model.

Step 7. Completely dry the skull surface. If using cyanoacrylate adhesive, apply a thin layer to the cannula. Using the cyanoacrylate, insert the brain cannula through the skull to the stereotaxic correct depth. To facilitate precise placement of the cannula, the tab on the side of the cannula can be attached to the electrode holder of a stereotaxic instrument (e.g., from Stoelting Co.). Alternately, this tab may be removed (e.g., using a heated scalpel) and the cannula placed by hand.

V. Longer Infusion Periods Using a Single Brain Cannula

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 midscapular region of the back, 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 at 5-10 cm anterior to the apert pump and remove the pump from the incision.

Step 3. Attach a new, fully-loaded pump with flow moderator in place to the incision site and then replace the cannula and trim the excess.

VI. Resources

StereotaxicAtlases


StereotaxicApparatus

Instruments that facilitate the stereotactic placement of ALZET Brain Infusion Kits are available from: Stoelting Co. 620 Wheat Road, Wheatfield, IL 60189 Tel.: (866) 860-9775 Fax: (630) 860-9775 email: info@stoelting.com

CannulaHolders

Cannula holders designed to fit the removable cannula tag of all ALZET Brain Infusion Kits are available from DURECT, Cannula Holders, 10240 Bubb Road, Cupertino, CA 95014 Tel.: (408) 865-1406 Fax: (408) 865-1406 email: info@p1tec.com

StainlessSteelScrews

Small stainless steel screws (size #0-40, 1/8” in length with a fillister head) are available from Plastics One Inc. (see above).

CyanocrylateAdhesive

DURECT sells Locite 454 (order #0008681), a cyanoacrylate adhesive for affixing the cannula to the skull.

DentalCement

Dental Cement (part number 15458 or 15459) is available from Stoelting Co. (see above)

VII. Technical Support

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 shown include subcutaneous and intraperitoneal injection (via the external jugular vein), localized administration in the central nervous system (both intracerebral and intraventricular), and other special applications. To obtain a copy of the video, please contact: ALZET Technical Support DURECT Corporation 10240 Bubb Road Cupertino, CA 95014 Tel.: (408) 865-1406 Fax: (408) 865-1406 email: info@p1tec.com

DURECT website: www.durect.com

IX. Warranty

For a period of 12 months from date of shipment, DURECT warrants the ALZET Brain Infusion Kit 3 (“Product”) from defects in materials and workmanship and conforms to the applicable specifications, instructions and Specifications Sheets.

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 in lieu of all other warranties, express or implied, and no other warranties, express or implied, are made by DURECT. DURECT’s obligation under this warranty is limited to the replacement of Product that does not conform to the foregoing warranty. DURECT will not be liable for any special, indirect or consequential damages, damages for loss of use or profit, or other similar damages.

This warranty gives you specific rights, and you may have other rights which vary from state to state.

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