

- Ten brain infusion cannulae
- Ten catheter tubes
- Forty depth adjustment spacers
- One instruction sheet

BRAIN INFUSION KIT 2 3-5 mm

STERILE MATERIALS FOR 10 BRAIN INFUSIONS

FEATURING A HIGH STABILITY, LOW PROFILE CANNULA

REFER TO ENCLOSED SPECIFICATIONS

CAUTION:

- Not for use in humans
- Not for veterinary use
- For use in laboratory research animals only

DURECT™ DURECT Corporation, Cupertino, CA 95014

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ALZET® OSMOTIC PUMP BRAIN INFUSION KIT 2 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 2 is for use with ALZET pumps for local delivery of test solutions to the central nervous system (CNS). The ALZET Brain Infusion Kit 2 can be 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 2 permit continuous delivery of compounds for extended periods of time without the need for external connections or frequent handling of laboratory animals

This kit is for use in experimental animals only. It is not to be placed in animals used for food or food products. This kit is not to be used in humans.

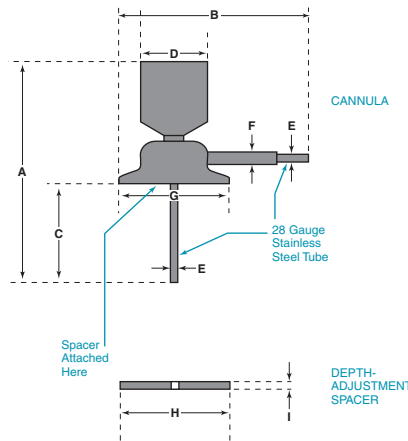
Direct access to the central nervous system (CNS) via a cannula implanted in the cranium is useful in experimental situations where a test compound has effects on the CNS but does not appreciably cross the blood-brain barrier. A test compound can be administered directly to the brain using this technique, 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 nature of the compound administered, intraven-tricular 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:

Sendelbeck SL and Urquhart J. Spatial distribution of dopamine, methotrexate, and antipyrine during continuous intracerebral microperfusion. *Brain Res* 1985, 328:251-258.

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



1. Brain Infusion Cannula

Material (tube) stainless steel
Material (elbow stop, flange) polycarbonate

Dimensions (± 0.1 mm)

A (height, overall)	11.3 mm
B (length, overall)	10.0 mm
C (height, tube)	5.0 mm
D (diameter, tab)	3.4 mm
E (outside diameter, tube/distal inlet)	0.36 mm
F (outside diameter, proximal inlet)	0.71 mm
G (diameter, base)	5.9 mm
Volume inside tube	0.32 µl

2. Height Adjustment Spacer

Material (spacer) polycarbonate

Dimensions (± 0.1 mm)

H (diameter, spacer)	5.9 mm
I (thickness, spacer)	0.5 mm

3. Catheter Tubing

Material (tube) polyvinylchloride (medical grade)

Dimensions

Length	15 cm (approx.)
Inside diameter	0.69 mm (± 0.08)
Outside diameter	1.14 mm (± 0.08)
Volume	3.74 µl/cm

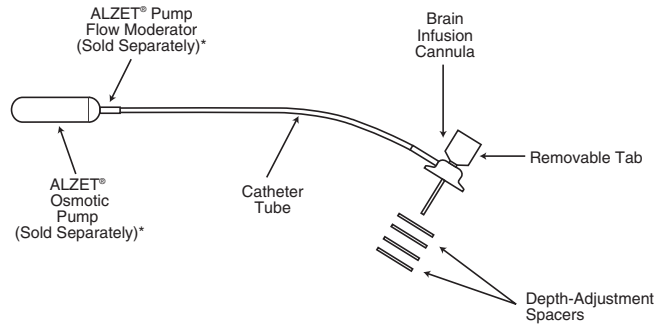
II. Checklist for Satisfactory Performance of the ALZET Brain Infusion Kit 2

- 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 techniques).
- For use with a catheter, ALZET pumps must be incubated in sterile saline at 37°C before implantation.** (Refer to Section III of this instruction sheet for complete instructions.)
- 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 ⁶⁰Co 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.



* 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 (408) 367-4036.

Step 1. Determine the correct stereotaxic coordinates for the target infusion site, and calculate the desired cannula length. DURECT has found that for intraventricular placement in Sprague-Dawley rats weighing approximately 250 g, a cannula depth of 4-5 mm below the skull is appropriate. 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.

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 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 cannula base using cyanoacrylate adhesive. The following table can be used to determine the number of spacers needed to achieve the depth desired. Note that the thickness of the spacers is 0.5 mm.

Number of Spacers Attached	Depth of Cannula Tube Remaining
0	5.0 mm
1	4.5 mm
2	4.0 mm
3	3.5 mm
4	3.0 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 project greater than 4 mm above the skull. This may make it difficult to close the scalp incision after cannula placement.

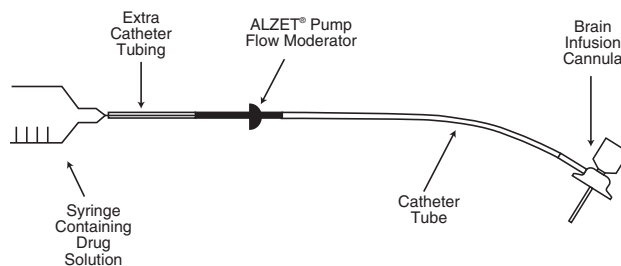
Step 3. After attachment of the spacers, verify the length of the remaining cannula tube. Also verify that the tube is straight and that it is at right angles to the bottom of the elbow stop and spacers. Should the cannula become contaminated during this procedure, soak it in an aqueous solution of 70% ethanol for several minutes. Before implantation, allow the ethanol to evaporate from the surface of the cannula and from the interior of the cannula tube.

Step 4. A 15 cm length of catheter tubing is included in this kit. This tubing can be used to attach the cannula 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 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 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 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 moderator. Check the attachment by gently pulling on the catheter. Neither end of this assembly should be loose or easily dislodged. If you are concerned about the security of this assembly, cement the catheter to the cannula and flow moderator with cyanoacrylate adhesive. The brain infusion assembly is now complete.

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.



Step 7. Fill the osmotic pump with the solution to be delivered, using the recommended procedure described in the ALZET osmotic pump instruction sheet. Ascertain 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 moderator in the filled osmotic pump. The pump and brain infusion assembly should now be completely filled and free of air bubbles.

Step 9. Incubate the filled brain infusion assembly with attached osmotic pump in sterile saline (0.9%) at 37°C for the time period recommended in the instructions for the pump 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 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 animal in a stereotaxic apparatus (e.g., from Stoelting, see Section VII).

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 the rounded end of a spatula, lightly scrape the exposed skull area and pat it dry. Scraping should remove the periosteal 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 midscapular area of the back of the animal for the osmotic pump. This pocket is created by using a hemostat to make a short subcutaneous tunnel from the scalp incision to the midscapular area and then opening and closing the hemostat to form the 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 onto the flank of the animal.**

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 determined in Section III, Step 1. With a pin vice 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 location. This hole will receive the cannula.

Step 5. The stability afforded by this brain cannula, with its wide base and low profile, 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 both the external portion of the cannula, and the dental cement that covers and secures the cannulation site. If you wish to place a small, stainless steel screw (available from Small Parts, Inc.), 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. Once the screw has been started into the skull, a turn or two is sufficient to secure it. The screw should extend approximately 2 mm above the skull.

Step 6. Insert the osmotic pump into the subcutaneous pocket, leading the catheter to the site for cannula placement. The osmotic pump 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. 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 the stereotaxically 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). Alternatively, this tab may be removed (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 8. 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 and 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 to clean tissue from the skull surface around the cannula site, and if the site is dry.

Step 9. Allow the cement to harden for four minutes and cut 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 into its cage. The animal requires no restraint or handling during the delivery period.

V. Verifying Cannula Placement & Patency

Upon sacrifice, verify the placement of the cannula and its patency as follows:

Step 1. Fix the brain with a suitable fixative (e.g., 4% formaldehyde).

Step 2. Remove the jaw and roof of the mouth of the animal and expose the floor of the brain.

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 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.

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 midscapular 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 fresh, fully-loaded pump with flow moderator 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:

Paxinos G. and Watson C. *The Rat Brain in Stereotaxic Coordinates* (4th ed.). Academic Press, San Diego, London, 1997.

Franklin K.B.J. and Paxinos G. *The Mouse Brain in Stereotaxic Coordinates*. Academic Press, San Diego, London, 1997.

Stereotaxic Apparatus and Cannula Holder

Heavy duty electrode holders fit the removable cannula tab and facilitate stereotaxic placement. These are available from:

Stoelting Co.
(Cannula holder part No. 51636)
620 Wheat Lane
Wood Dale, IL 60191
Tel.: (630) 860-9700
Fax: (630) 860-9775
email: physiology@stoeltingco.com
www.stoeltingco.com

David Kopf Instruments
(Cannula holder part No. 1776-AP-A or 1776-P1)
7324 Elmo Street
Tujunga, CA 91042
Tel.: (818) 352-3274
Tel.: (877) 352-3275
Fax: (818) 352-3139
email: sales@kopfinstruments.net
www.kopfinstruments.com

Pin Vice and Drill Bit

Holes in the skull can be drilled with a steel drill (number 56, part number O-HSD-56) and a small pin vice (part number O-PV-5). These are available from:

Small Parts, Inc.
13980 NW 58th Court
P.O. Box 4650
Miami Lakes, FL 33014-0650
Tel.: (305) 557-8222
Tel: (800) 220-4242
Fax: (305) 558-0509
email: smlparts@smallparts.com
www.smallparts.com

Stainless Steel Machine Screws

Small stainless steel machine screws (size #0-80, 1/8" in length with a fillister head, part number MX-0080-02 FL) are available from Small Parts, Inc. (see above)

Cyanoacrylate Adhesive

Durect sells Loctite 454 (product #0008670), a cyanoacrylate adhesive gel for affixing the cannula to the skull.

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 special applications for ALZET osmotic pumps. Surgical procedures shown include subcutaneous and intraperitoneal implantations, intravenous infusion (via the external jugular vein), localized administration in the central nervous system (both intraventricular and intrathecal), gastrointestinal delivery, and other special applications. To obtain a copy of the video, please contact:

ALZET Technical Support
DURECT Corporation
10240 Bubb Road
P O Box 530
Cupertino, CA 95015-0530

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

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 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, 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, INCLUDING WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE, WHICH ARE HEREBY EXCLUDED. IN NO EVENT SHALL DURECT BE LIABLE FOR ANY SPECIAL, INCIDENTAL, INDIRECT OR CONSEQUENTIAL DAMAGES, HOWEVER CAUSED, ON ANY THEORY OF LIABILITY. DURECT'S LIABILITY WITH RESPECT TO THE PRODUCT SHALL IN NO EVENT EXCEED THE AMOUNT PAID BY THE CUSTOMER FOR THE PURCHASE OF THE PRODUCT GIVING RISE TO SUCH LIABILITY.