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 for the surgical implantation procedure (Refer to Sections III and IV of this instruction sheet for correct filling and implantation techniques).

3. Ensure that the vehicle/solvent used is compatible with polyethylene glycol solution.

4. When using the Alzet Brain Infusion Kits, 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 stereotactic 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 stereotactic location.

1. Correct placement and patency of the cannula can be verified at the termination of infusion by injecting dye through the cannula.

2. Attachment of a catheter to an osmotic pump does not alter its drug delivery rate.

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 figures 1-4, which should be done prior to the animal being filled and before Alzet osmotic pumps. To prepare the brain infusion assembly, perform the following steps:

Step 1. Determine the correct stereotaxic coordinates for the target infusion site, and calculate the desired cannula length. For example, a cannula length of approximately 2-3 mm is appropriate for infusion into the lateral ventricles (5 mm above the skull). This may make it difficult to close the scalp incision after cannula placement.

Step 2. Without modification, the L-shaped cannula included in this kit will penetrate approximately 3 mm below the surface of the skull. Depending on the size of the skull, skull thickness and desired site for infusions, this depth may need to be altered. Spacers are included in this kit to allow you to alter the cannula length. To do so, slide the desired number of depth adjustment spacers onto the cannula tube and glue them in place to achieve the desired depth. The following table can be used to determine the number of spacers needed to achieve the desired depth.

Note: The dimensions in this table are based on DURECT’s experience with brain infusion. They may not be appropriate in all particulate applications. DURECT recommends that investigators using the Alzet Brain Infusion Kit 3 determine the coordinate, site and depth adjustment which provide optimal results in their particular brain infusion model.

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 not bent or crushed at the end of the spacers 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.

Step 4. A 15 mm 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, determine 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. In this way, there will be approximately 6 cm of the lumen of the catheter and 3 cm of the cannula tube, to allow free movement of the animal’s head and neck.

Step 5. Cut the catheter tubing to the length determined in Step 4. Then attach one end of the tubing to the cannula and the other end to the Alzet pump flow modifier. Note: When attaching a catheter to the pump: 200 μl & 2 ml Models: Remove the translucent cap from the tube-inlet flow modifier, revealing a short stainless steel tube protruding from the white flange. 100 μl Models: Remove and discard the white plastic 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 transparent cap on the flow modifier. Check the attachment by gently pulling on the catheter. Failure of this attachment indicates that the flow modifier is not securely attached. If you are concerned about the security of this assembly, cement the catheter to the cannula tube and flow modifier with cyanoacrylate adhesive.

The brain infusion assembly is now complete.
Step 1. Fill the brain infusion solution with the delivery to be delivered. Do so by attaching a Luer Lock fitting to the solution to be delivered to the remaining catheter tubing and connect the tubing to the free end of the flow modulator.

Step 2. Fill the osmotic pump with the solution to be delivered, using the recommended procedure described in the ALZET osmotic pump instruction sheet. Adjust the delivery rate to be greater than or equal to the volume specified on the instruction sheet for that model.

Step 3. Place the flow modulator in the filled osmotic pump. The pump and pump assembly should now be completely filled and free of air bubbles.

Step 5. Incubate the filled brain infusion assembly with attached stereotactic pump in sterile saline (0.9%) at 37°C for the time period recommended in the instructions for the pump model being used. This is to make sure that the ALZET osmotic pump is used as a catheter, as it ensures that the pump is pumping continuously prior to implantation. It is also a good idea to wash the cannula with 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.

Step 11. The anesthesia of the mouse or rat (e.g., with an intraperitoneal injection of a solution of pentobarbital, 40-50 mg/kg) and fit the animal in a stereotaxic apparatus (e.g., from Stoelting, see Section V).

Step 12. (Shave and wash the scalp). Slit the parietal bone of the skull with a sharp surgical blade. Make sure to keep the skin edges apart.

Step 13. Identify the bone sutures, juncions, bregma and lambda. With these as reference points, determine and mark the location for the cannula placement using the stereotaxic coordinates determined in Section III.

Step 2. Make an incision and expose the skull. With the rounded edge of a scalpel, sharply scrape the exposed skull area and remove any bone. Scrapping should remove the periosteal connective tissue which adheres to the skull, permit accurate placement of the dental cement which is used later to secure the cannula.

Step 6. 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 sharp surgical blade to incise the subcutaneous tissue. The incision should be made perpendicular to the surface of the skin.

Step 7. Completely dry the skull surface. If using cyanoacrylate glue, apply a thin layer to the cannula. Using the midline hole, insert the brain cannula through the skull to the stereotaxically correct location. This hole will receive the cannula.

Step 8. Insert the cannula into the osmotic pump subcutaneously, leading the catheter up to the site for cannula placement. 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 9. Attach a fresh, fully-loaded and primed pump. The pump fitting should be replaced by a new, fully-loaded and primed pump.

Step 15. Apply a thin layer of Dental Adhesive to the cannula. Make sure the cannula is attached to the electrode holder of a stereotaxic instrument (e.g., from Stoelting). Alternatively, this tab may be removed with a small, stainless steel machine screw (size #0-80, 1/8" in length with a round tip); Cannula holder 2 (order #0008861). The stability afforded by this brain cannula, with its wide base and low profile, may make placement of a stabilizing screw unnecessary.

Step 16. 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 to be used 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 cannula site. If you wish to place a small, stainless steel screw, drill a second hole part-way through the bone adjacent to the first hole and 4 mm to the left 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 below the skull.

Step 17. 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 to be used 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 cannula site. If you wish to place a small, stainless steel screw, drill a second hole part-way through the bone adjacent to the first hole and 4 mm to the left 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 below the skull.

Step 18. If using cement; cover the cannula, the entire cannula site. The cement should be completely dry before being removed.

V. Verifying Cannula Placement and Patency

Step 1. The catheter and slowly inject a dye (e.g., Evans Blue) through the catheter toward the cannula. Expose the tip of the catheter and examine the dye stains to confirm its placement. Alternatively, after the catheter is removed, the brain can be fixed, frozen, and sectioned to confirm cannula placement.

Step 2. The catheter and slowly inject a dye (e.g., Evans Blue) through the catheter toward the cannula. Expose the tip of the catheter and examine the dye stains to confirm its placement. Alternatively, after the catheter is removed, the brain can be fixed, frozen, and sectioned to confirm cannula placement.

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.: (800) 865-9775
  Fax: (630) 865-9775
  email: info@stoeltingco.com
- Stoelting Co.
  7941 S. Ogden Ave.
  Bridgeview, IL 60455
  Tel.: (708) 352-3274
  Fax: (708) 352-3275
  email: sales@stoeltingco.com
- Stoelting Co.
  www.stoeltingco.com

Cannula Holders

Cannula holders designed to fit the removable cannula tubings of all ALZET osmotic pumps are available from DURECT. Cannula holder 1 (order #0008860); Cannula holder 2 (order #0008861).

Pin Vise and Drill Bit

Holes in the skull can be drilled with a steel drill bit (1/64”) and a small pin vise with steel drill bit (1/64’). These are available from:

- Plasticine Inc.
  6591 Merriman Rd
  Roselle, IL 60172
  Tel.: (540) 772-1168
  email: info@durect.com

Stainless Steel Machine Screws

Small stainless steel machine screws (size #0-40, 1/8” in length with a flatted head) are available from Plasticine (see above).

Cyanoacrylate Adhesive

DURECT sells Locite #54 (item #0009670), a cyanoacrylate adhesive gel for affixing the cannula to the skull.

Dental Adhesive

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 techniques and special applications for ALZET osmotic pumps. Surgical Procedures Available on Video

Surgical Procedures Available on Video

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www.durect.com
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web site:

IX. Warranty

For a period of 12 months from date of shipment, DURECT warrants the ALZET Brain Infusion Kit #3 (‘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 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, without limitation, the implied warranties of merchantability and fitness for a particular purpose, which are hereby excluded. In no event shall DURECT be liable for any special, indirect, incidental, or consequential damages, however named, including, without limitation, the loss of any actual or expected revenue, profits, or anticipated savings, or the costs of replacement goods and services, and all expenses of whatever kind or nature incurred by any party in connection with the purchase or use of the Product. This warranty gives you specific legal rights, and you may also have other rights which vary from jurisdiction to jurisdiction.

DURECT Corporation
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