
 University of Zurich Institute of Laboratory Animal Sciences	Standard Operating Procedure SOP	Page 1 of 6
Date: 14.11.2022	Implantation of osmotic minipumps	LTK-RES-4-E-EN Version: E
This SOP replaces: Version: D		
Reason for Change: Response to veterinary office		
Related SOPs:	SOP-LTK-TRT-13 Isoflurane anesthesia SOP-LTK-TRT-18 Injection anesthesia SOP-LTK-RES-5 Scoring and withdrawal criteria of i.c. tumors SOP-LTK-RES-6 Bioluminescent in vivo imaging SOP-LTK-TRT-43 Buprenorphine analgesia SOP-LTK-TRT-19 Tail Bleeding SOP-LTK-RES-3 Stereotactic injection SOP-LTK-Res-41 Scoring post-application of binder proteins	
Indication of Use:	Continuous intracranial delivery of biologically active (cytokines, small molecules, peptides, antibodies, chemotherapy) and inactive (tracers) substances via osmotic minipumps	
Aim of SOP:	This procedure describes how to implant Alzet osmotic minipumps for the continuous intracranial (i.c.) application of liquids containing biologically active or inactive compounds into anatomical structures at defined three dimensional coordinates	
Distribution:	<ol style="list-style-type: none"> 1. Server 2. Animal Facility 3. Group vom Berg 	
Attachments:		
Generated at: 14.11.2022	Checked and approved at: 14.11.2022	
by: Michal Beffinger	by: Johannes vom Berg	

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Responsible Persons: Any person with Module 1 and registered on a particular animal permit

Method: microsurgery

Principle of Method:


ALZET pumps operate because of an osmotic pressure difference between a compartment within the pump, called the salt sleeve, and the tissue environment in which the pump is implanted. The high osmolality of the salt sleeve causes water to flux into the pump through a semipermeable membrane which forms the outer surface of the pump. As the water enters the salt sleeve, it compresses the flexible reservoir, displacing the test solution from the pump at a controlled, predetermined rate. Because the compressed reservoir cannot be refilled, the pumps are designed for single-use only.

The **ALZET Brain Infusion Kits** are designed specifically for use with ALZET pumps for targeted delivery to the central nervous system. They can be used in two ways:

Infusion into the cerebral ventricles exposes a wide variety of brain regions to the infusate via the cerebrospinal fluid which bathes the brain.

Direct microperfusion of discrete brain structures results in localized distribution of infusate in the target tissue.

By fixing the head in all three dimensions, exact anatomical regions within the brain can be reached by using manipulator arms. Stereotactic coordinates for specific structures can be found in stereotactic atlases, such as **“The Mouse Brain in Stereotaxic Coordinates”** by **George Paxinos, Keith B. J. Franklin**”

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Material to be used:

2 weeks – Alzet® #1002 0.25 µl/h
2 weeks – Alzet® #2002 0.5 µl/h
4 weeks – Alzet® #2004 0.25 µl/h
6 weeks – Alzet® #2004 0.15 µl/h
Alzet® Brain infusion Kit III #0008851
All suitable for mice heavier than 10g body weight (6 week old C57/BL6 usually weighs between 17-23 g)
Surgical tools: scalpel, scissors (small and large), forceps
Betadine Iodine solution
Sterile cotton swabs
Dental cement / high-viscosity acrylamide glue
0.4 mm nylon / metal clamps / tissue glue (Indermil®, Henkel®)
Electrical heating mat, small animal electrical hair trimmer
Vit A eye ointment / Humigel

Material acquisition:
Alzet is distributed by Charles River


Calibration:
Pumps are ready to use, variation is lot dependent, so only use pumps from the same lot (all info in the accompanying package insert)

Storage of Material:
Pumps and infusion kit at RT

Machine:
Regular small animal stereotactic frame e.g. from DKI or Stoelting


Safety:

1. General rules for working with sharp tools (scalpels, syringes, scissors) have to be followed.
2. Only in the case of chemotherapeutic agents and human cytokines additional biosafety rules have to be obeyed
3. Follow the rules of the animal house

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Method Description:

1. Sterilize all material, tools and solutions prior to surgery
2. Adjust catheter to 1.5-2 cm length
3. Fill pump, catheter and needle with compound solution according to technical data sheet, make sure that no air bubbles are trapped and assemble the implant
4. Incubate implant in PBS at 37°C for 6-24h (depending on pump model, see respective technical data sheet) to start the osmotic process (“priming”)
5. Animals are anesthetized (**following SOP-LTK-TRT-18 Injection anesthesia**), shaved (use a small animal hair trimmer, shave the head area) and mounted onto a stereotactic frame (**SOP-LTK-RES-3 Stereotactic injection**). The skin on the head is disinfected, eyes are being protected from drying out by application of eye ointment.
6. The scalp is incised by 1 cm long median-sagittal cut.
7. The skull is dried and cleaned of periosteal connective tissue with a sterile cotton swab
8. Take a closed arterial clamp and – starting from the skin incision - form a little pocket for the pump at the height of the scapulae by opening the clamp carefully.
9. Find bregma (the intersection of the coronal and sagittal sutures)
10. Place the drill over bregma, move 2 mm lateral and 1 mm frontal, drill a hole until you reach the dura.
11. Place the cannula of the implant (Alzet brain infusion Kit III) at the desired Z position in the head (usually 3 mm below the dura) and fix it with dental cement (or high-viscosity acrylamide glue) on the skullcap
12. Put the pump into the skin pocket at the neck over the scapulae, this way the mouse has full freedom of movement.
13. Suture the skin incision using a 0.4 mm nylon or metal clamps and/or tissue glue (Indermil®, Henkel®)
14. Apply analgesia and antidote in one mix according to **SOP-LTK-TRT-18 Injection anesthesia**
15. Move animal into the wake up cage (a regular cage, half of it placed on a 37°C electrical heating mat, covered with a surgical cloth), only put fully awake animals back to the housing cage.
16. Check for postoperative complications and signs of pain after 1 h, apply analgesia (**SOP-LTK-TRT-43 Buprenorphine analgesia**) s.c. after 4 hours and repeat every 4-6 hours during the light phase of the light cycle. During the dark phase of the light cycle administer Buprenorphine via drinking water (**SOP-LTK-TRT-43 Buprenorphine analgesia**). Check the next day and re-apply analgesia. Keep animals on analgesia for at least 36 hours after surgery. Repeat this cycle of checks every 4-6h during the day plus the drinking water in the night until you do not detect pain signs anymore; for up to 3 days. Monitor mice according to **SOP-LTK-RES-5 Scoring and withdrawal criteria of i.c. tumors** or **SOP-LTK-RES-41 Scoring post-application of binder proteins** (if no tumors are implanted)
17. **For experiments that exceed the indicated maximal working time of the pump model used** (check technical data sheet and pump specifications), **explant pump:** Perform steps 1 and 5 of this SOP, then make a 1 cm midline incision on the scalp, carefully lift the disc of the brain infusion kit (it might be necessary to loosen the

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cement / glue using the forceps) and slowly take out the infusion cannula. Now remove the actual pump together with the silicon tubing. Clean contact area where the disc was glued to the skull and with a sterile cotton swab and cover burr hole with bone wax. Then follow steps 13 - 16 of this SOP.

Controls:
 After pump removal, check remaining volume and check whether or not tubing was clogged

Factors influencing outcome:
 Low temperature reduces the osmotic process - which is designed for 37°C - also during surgery keep pumps at 37°C (e.g. in a small incubator), remove them only just before implantation

Pain management:

SOP-LTK-RES-4 Implantation of osmotic minipumps
 SOP-LTK-TRT-18 Injection anesthesia

Euthanasia ← *if yes*

Re-apply analgesia s.c. ← *if yes*

SOP-LTK-TRT-43 Buprenorphine analgesia (for up to 3 days, s.c. 0.1 mg/kg BW)
 SOP-LTK-RES-5 Scoring and withdrawal criteria of i.c. tumors
 SOP-LTK-Res-41 Scoring post-application of binder proteins (in case mice are not tumor bearing)

Anesthesia

↓

Implantation of minipumps

↓

Anesthesia antidote, s.c., containing buprenorphine analgesia

↓ *1h post buprenorphine*

Check for pain symptoms

↓ *if no*

Check for pain symptoms every 4-6 hours


↓ *dark phase*

Buprenorphine analgesia drinking water o/n

↓ *next morning*

Buprenorphine analgesia s.c.

Documentation:
 Lab book and Score sheet according to **SOP-LTK-RES-5 Scoring and withdrawal criteria of i.c. tumors**
 The mice have to be placed in the respective experiment and project in iRATS. The actual severity has to be recorded in iRATS at the end of the experiment for each mouse.

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Problem management:

Report any adverse event to your supervisor or vet, In case there is arterial bleeding (strong and pulsating bright-red bleeding), euthanize the animal by immediate overdose of injection anesthesia (**SOP-LTK-TRT-18 Injection anesthesia**)

Literature:

Intratumoral IL-12 combined with CTLA-4 blockade elicits T cell-mediated glioma rejection. Vom Berg J, Vrohling M, Haller S, Haimovici A, Kulig P, Sledzinska A, Weller M, Becher B. J Exp Med. 2013 Dec 16;210(13):2803-11. doi: 10.1084/jem.20130678.