

Protocol: Ancrod Pump

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Preparation of ALZET Osmotic Ancrod Pumps for Subcutaneous Implantation

Reagents/Supplies

1. Mini-osmotic pumps (ALZET 1007D, 2002). A filling needle is included in the box with osmotic pumps.
2. Reagent for osmotic pump infusion; Ancrod purchased from NIBSC, Cat # 74-581; store at - 20°C
(https://www.nibsc.org/products/brm_product_catalogue/detail_page.aspx?catid=74/581)
3. Sterile Hyclone Water (GE Healthcare Life Sciences, SH30529.01, endotoxin-free) and Saline
4. Plastic tubes for dissolution of the reagent (15 ml; sterile) or incubation of osmotic pumps (50 ml; sterile)
5. Eppendorf tubes to prepare solution for individual mice (1.5 ml, sterile)
6. 1 cc syringe (sterile) to inject solution into osmotic pumps

Pump Information:

Alzet pump model	1007D	2002
Duration (days)	7	14
Pump rate (μ l/h)	0.5	0.5
Mean fill volume (μ l) *	100	200

* mean fill volume may differ with different lot #.

Ancrod preparation

1. Design the experiment and determine the length of the infusion and the infusion rate.
2. Example) Alzet 2002-0.5 $\mu\text{l/hr}$ x 14 days, Mean fill volume = 213 μl
Ancrod concentration in vivo: 3 IU/day
: Pump 0.52 $\mu\text{l/hr}$ x 24 hrs = 12.48 $\mu\text{l/ day}$
: (0.125 IU/hr) * (hr/0.52) = 0.24 IU/ μl

In case of 4 pumps: 225 μl of sterile hyclone water x 4 = 900 μl

For 3.00 IU/day:

Ancrod (NIBSC standard): 55 IU/ampoule.

: 4 ampoules = 55x4 = 220 IU/900 μl sterile hyclone water = 0.244 IU/ μl

Pumps: 12.48 $\mu\text{l/day}$ x 0.244 IU/ μl = 3.05 IU/day

3. Equilibrate ancrod vial to room temperature before opening.
4. Add the calculated volume of sterile hyclone water (endotoxin free) into the plastic tube with the lyophilized ancrod, cap and mix thoroughly until the solution becomes clear.
5. Prepare ancrod solution under a cell culture hood.

Osmotic Pump Filling

Follow ALZET pump instructions:

1. Pumps are supplied in two separate parts: the main body of the pump and the flow regulator. Open only the number of pumps and flow regulators needed for the study, as these cannot be stored once opened. Record lot # in your note. ALWAYS USE GLOVES! Natural oils from your hands may damage the exterior of the pump casings.
2. Use mean fill volume provided by alzet or calculate the volume as follow:
Weigh each pump (main body and flow regulator) individually, and note the weight to 4 decimal places (e.g. 1.1018 grams). This (called as “empty weight”) will be used to calculate the fill volume. Place each weighed pump in weight boat marked with the mouse number.
3. Attach the filling needle to a 1 cc syringe and carefully draw up ancrod solution from Eppendorf tube. It is important to minimize the air drawn into the syringe along with the ancrod solution.
4. Carefully remove all bubbles from the syringe and invert with the needle aimed at the floor. Keep the needle/syringe in this position to prevent the introduction of bubbles into the pump.
5. Gently insert the filling needle/syringe into the pump body. Advance the tip of the needle into the pump. Ensure the tip of the needle does not rest tightly on the bottom of the pump.
6. Fill the pump slowly. Notice the dark shadow inside the pump indicating the fluid level. Watch this level rises. STOP filling the pump as soon as you see a bead of fluid rises out of the pump body. Carefully remove the needle/syringe and draw up the excess fluid that came out of the pump body.
7. Insert the flow regulator into the body of the pump. Make sure the regulator is seated tightly against the pump body. As you insert the regulator into the pump body, you may notice some fluid leaking out the opening of the flow regulator. THIS IS NORMAL. Carefully blot up all extra fluid that might have leaked during the regulator placement.
8. Weigh the filled pump. This is now marked as “fill weight”. Filling volume = “fill weight” - “empty weight”. Assume that 1 fill volume of fluid = 1 fill volume of weight. Compare this calculated volume to the mean fill volume indicated in the Instruction for the lot #. See the following example for calculation.

Mouse ID	Empty Weight (g)	Fill Weight (g)	Actual Fill volume = (Fill Weight - Empty Weight) x 1000	% Fill = (Actual Fill Volume/ Mean Fill Volume*) x 100%
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1	1.2245	1.4674	$= (1.4674 - 1.2245) \times 100 = 242.9$	$= (242.9/243) \times 100\% = 100\%$
2	1.2437	1.4934	$= (1.4934 - 1.2437) \times 100 = 249.7$	$= (249.7/243) \times 100\% = 103\%$

9. Place the filled pump into the labeled test tube with the regulator head facing UPWARDS. Add enough sterile saline to cover the pump. Pumps should be kept in test tubes with saline until ready for use.

10. Place test tubes in a 37 °C incubator. Incubate pumps overnight (at least 12 hours) to allow partial priming. Pumping of ancrod will start 24 hours after the implantation surgery. This allows mice to recover from the surgery prior to the potential stress of ancrod infusion. If a second pump is needed to continuously infuse ancrod after removal of the first pump, it must ensure that pumping has already started before the surgery. This requires that pumps are incubated in sterile saline at 37 °C overnight.

Surgical Implantation of Mini-Osmotic Pumps

Equipment for Pump Implantation:

1. Sterile Pack: surgical scissors, straight hemostats, forceps, wound clip kit, drapes, cotton swabs, gauze squares
2. Sterile gloves
3. Surgical mask
4. 3 conical tubes: Betadine, 70% ethanol, Sterile water.
5. Pumps for implantation
6. Shaving implement
7. Antiseptic handrub
8. Topical 4% Lidocaine
9. Isoflurane vaporizer
10. Induction chamber
11. Nose cone
12. Oxygen
13. 2 charcoal canisters

Preparation of Autoclave Pack

1. Put gauze and cotton swabs on a surgical drape and fold corner to corner and put an autoclave tape on it. Wrap the pack with another drape and tape with autoclave tape.
2. Put all the surgical tools (scissors, hemostat, forceps, staples and stapler) in the sterilization pouches and seal.

Autoclave both the packs in dry cycle.

Preparation of Vaporizer

Fill Isoflurane and have induction box in the prep area and nose cone in the clean area of laminar flow hood.

Preparation for Surgery

1. Set up betadine, 70% ethanol, sterile water, bead sterilizer, hand cleaner, swabs, gauze, and the pumps in the laminar hood.
2. Surgeon puts on the mask and gown, and then opens up the outer drape in the laminar hood with clean hands. Put on sterile gloves and open the inside pack.
3. Set up sterile drape with nose cone in the laminar flow hood.

Surgical Procedure

1. Place mouse in induction chamber.
2. Once anesthetized, place mouse in laminar hood with nosecone. Mouse head points toward your dominant hand.
3. Swab and wipe area with betadine three times, and then 70% ethanol.

3. Use surgical scissors to make a 1 cm incision behind the ear over the shoulder blade of the front leg. This incision should be perpendicular to the tail. Use care to cut only the skin but not the underlying tissues.
4. Wipe incision area once (toward the tail end of the mouse) with folded kimwipes soaked with 70% ethanol. Do not wipe repeatedly.
5. Use one hand to hold forceps to open the incision, and use another hand to hold a straight hemostat to make a subcutaneous tunnel under the skin.
6. Advance the tip of the hemostat toward the tail. Create a pocket for pump. This is accomplished by carefully opening the jaws of the hemostat under the skin to open up a pouch. Pull the hemostat back out of the incision as you close the jaws back together.
7. Insert pump with the regulator head first into the incision (Pointed toward the tail end of the mouse).
8. Gently push the pump completely into the pocket. There should be enough skin free to close the wound with no tension or stretching of the skin needed.
9. Once the pump has been inserted, firmly pinch both sides of the incision and staple the incision. Inspect the incision site to ensure that there is complete closure of the wound.
10. Apply Topical 4% Lidocaine cream with a clean swab.
11. Return the mouse to its cage, and repeat above steps for the next mouse.
12. Place surgical instruments into a bead sterilizer for 10 seconds between mice. Dip in sterile water, be sure to allow instruments to cool before use. Clean hands with Antiseptic handrub between mice.
13. Monitor all mice until full recovery is achieved. Fill the post operation card. Monitor mice closely within 7 days after the surgery.