

Subcutaneous Infusions of Angiotensin II with ALZET Osmotic Pumps

Supplies Needed:

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| 1. Osmotic pumps (ALZET 2004) | 5. Sterile 0.9% saline |
| 2. Angiotensin II (Sigma A-9525, 10mg vial) | 6. Ice |
| 3. 5 cc syringe | 7. Filling needle (included with the pumps) |
| 4. Plastic culture test tubes (~ 16x100mm) | |

Dosage Calculations:

1. Weigh mice immediately before calculating doses. Mean the weights of mice per group.
2. Determine the length of the infusion, either 2 weeks or 4 weeks.
3. For calculating the proper amount of AngII, assume that mice will gain 1 gram of weight per week if on a western diet, or 0.5 grams per week if on normal chow diet.
4. Calculate the estimated weight of the group of mice based upon the expected weight they will have gained at the midpoint of the infusion. (i.e. If your group weigh is initially 25 grams, and mice are on a western diet and will be infused with AngII for 4 weeks, then estimate that their weights will be approximately 27 grams at the midpoint of the infusion.)

25 grams + (1 g/wk for 2 weeks) = 27 grams at midpoint of 4 wk study

Base dosing calculations on this midpoint weight, so mice will be overdosed for the initial 2 weeks of the study and under dosed for the last 2 weeks of the study. An example for two different weights of mice are shown in the table below.

Initial Weight	Midpoint Weight	Desired Dose of AngII	Pump Rate ml/hr	Pump Fill Volume	Number of Pumps	Final Volume of Saline	AngII (mg)
25 grams	27 grams	1000 ng/kg/min	0.25	242 : l	10	3 ml	19.29 mg
30 grams	32 grams	1000 ng/kg/min	0.25	242 : l	10	3 ml	22.86 mg

5. Calculate the proper amount of AngII to use based on: **1) the midpoint weights of the mice, 2) the pump fill volume, 3) the mean pump rate, and 4) the number of pumps to be filled.** Use attached worksheet to calculate proper dosing.
6. Pumps are supplied in two parts, the main body of the pump that holds the infusate, and the flow regulator. Open only the necessary number of pumps for the study, as these cannot be stored once opened. **NOTE: ALWAYS USE GLOVES! The natural oils from your hands will damage the exterior of the pump casings.**
7. Gently assemble the pumps, placing the flow regulator into the body of the pump. **NOTE: Make certain that the flow regulator is properly aligned when inserting into the pump body. The pumps should be empty at this point.**
8. Weigh each pump individually, noting the weight to 4 decimal places. (i.e. - 1.1018 grams). This will serve to help calculate the fill volume. Place each weighed pumped upside down in the test tubes.

Dissolution of AngII and Filling Pumps:

1. Remove AngII from freezer and allow to come to room temperature.
2. Chill saline prior to use, maintain on ice during filling of pumps.

3. Take appropriate number of AngII vials, and log the lot numbers in your notes.
4. Weigh (APPROXIMATELY ACCURATELY) Ang II and transfer the lyophilized powder to a **plastic** culture tube.
5. Add the measured volume of chilled saline, cap and vortex thoroughly to mix.
6. Attach the filling needle to the 5 cc syringe and carefully draw up all the mixture, take care to minimize the air drawn into the syringe along with the mixture.
7. 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.
8. **USE CARE** when inserting the filling needle/syringe into the pump body. Advance the tip of the needle into the pump until you can feel the bottom, then carefully withdraw the needle just slightly (~2 mm) so that the tip of the needle does not rest directly on the bottom of the pump.
9. Begin filling the pump **slowly**. It is possible to see a dark shadow inside the pump indicating the fluid level. Watch this level rise as you continue to fill the pump. **STOP** filling the pump as soon as you see a bead of fluid rise out of the pump body, around the needle. If you overfill, and have a large bead of fluid, carefully remove the needle/syringe and draw up the fluid. This will save solution volume.
10. Set the needle/syringe aside in the ice bucket, making sure to keep the needle pointed towards the floor.
11. Insert the flow regulator back 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 on the flow regulator. **THIS IS NORMAL. Do not block this outflow when inserting the regulator into the pump body, as this will build pressure within the pump that might rupture the internal bladder.** Carefully blot up all extra fluid that might have leaked during filling or regulator placement.
12. Re-weigh the pump. This is now marked as the fill weight. After all pumps are filled, go back and subtract the pump weight from the fill weight. This will be the approximate weight of the fluid remaining in the pump. (Assume that 1 cc of fluid = 1 mg of weight) Therefore, the remaining weight in the pump is also the fill volume (see chart below).

Pump #	Pump Weight	Pump Fill Weight	Fill Volume (pump fill weight - pump weight)
1	1.2245 g	1.4674 g	242.9 ml
2	1.2231 g	1.4527 g	229.6 ml
3	1.2250 g	1.4669 g	241.9 ml

13. After weighing the pump, return to the test tube, this time regulator UPWARDS. Add enough sterile saline to cover the tips of the pumps by at least 5 mm. **Pumps should be kept in test tubes with saline until ready for use.**
14. Place all test tubes in the incubator at 37 °C for 24 hours prior to implantation. Normally the pumps should incubate for 48 hours before they will begin to release AngII at the proper flow rates and doses. However, this 24 hour incubation will allow the pumps to partially prime. They should not begin releasing AngII for an additional 24 hours after implantation. This will allow the mice 1 day to recover from the surgery of implantation, prior to the potential stress of AngII infusion.

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