

## Preparing ALZET Osmotic Pumps with Angiotensin II for Subcutaneous Infusions

Supplies needed:	
1. Mini-Osmotic pumps (ALZET 2004) or Micro-Osmotic pumps (ALZET 1004)	4. Plastic test tube
2. Angiotensin II (stored at -20°C)	5. Sterile saline
3. 1 cc syringe	6. Filling needle (included with the pumps)
	7. Eppendorf Tubes

### Dosage Calculations:

1. Weigh mice immediately before calculating doses.
2. Determine the length of the infusion, either 2 weeks or 4 weeks (most infusions are 4 weeks long) and dose of AngII (i.e. 500ug or 1000ug)
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. Use Microsoft Excel worksheets as templates to calculate pump fill percentages (AD Lab has own template - file pathway: <Z:\Protocols and forms\MOUSE\Pump implantation\pump calculation sheet>)
5. Pumps are supplied in two parts, the main body of the pump that holds the solution, 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.**

6. 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 in weigh boat marked with appropriate mouse number.



Figure 1a. Ang II Pump with flow regulator

<http://www.alzet.com/products/specifications.php>



Figure 1b. Flow regulator

### Dissolution of AngII and Filling Pumps:

1. Remove AngII from freezer and allow thawing to room temperature before opening.
2. Take appropriate number of AngII vials to obtain the total amount of AngII needed for dissolution, and log the lot numbers in your notes.
3. Weigh out appropriate amount of Ang II and transfer the lyophilized powder to a **plastic** culture tube (**do not use glass – solutions of AngII have a high affinity for glass**).
4. Add the measured volume of saline, cap and invert to mix.
5. Pipette the appropriate amount of Ang II mix and then appropriate amount of saline into an eppendorf tube labeled with the mouse number. Refer to Quattro Pro worksheet for appropriate volumes.
6. Attach the filling needle to the 1 cc syringe and carefully draw up all the mixture from the eppendorf tube, 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 taking care that the tip of the needle does not rest directly on the bottom of the pump.

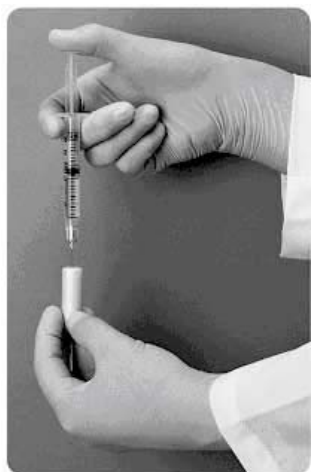


Figure 2a. Diagram exhibits filling of Alzet pumps.

[www.alzet.com/products/filling.php](http://www.alzet.com/products/filling.php)

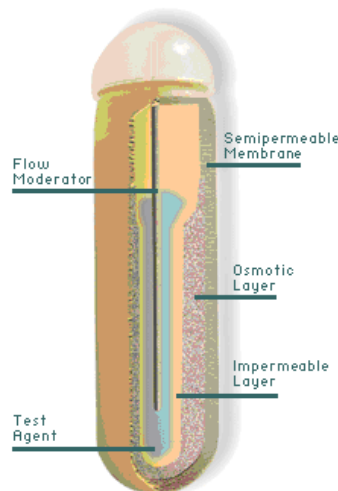


Figure 2b. Inside of Alzet pump.

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. 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.
11. 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). Compare this to the fill volume on the pump insert.

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

12. After weighing the pump, return to the test tube, with regulator facing UPWARDS. Add enough sterile saline to cover the pumps. **Pumps should be kept in test tubes with saline until ready for use.**

13. Place all test tubes in the incubator at 37 °C for 24 hours prior to implantation. Normally, the pumps will begin to release AngII at the proper flow rates and doses after 48 hours of incubation. 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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