In Vivo Perfusion System Instruction Sheet

Thank you for purchasing the AutoMate *In Vivo* Perfusion System. We trust you will find our equipment easy to use and efficient for perfusing small rodents. There are five sections to this instruction sheet:

1) Expectations — a few guidelines on perfusion with our system
2) References — published literature on perfusion and anesthetics
3) Assembly Instructions — the correct order for assembling our product
4) Perfusion Technique — protocol for small animal perfusion
5) Other Useful Information

Expectations

1. AutoMate Scientific *In Vivo* gravity perfusion systems are designed for perfusing small rodents (Mice: AutoMate #11-140, Rats: AutoMate #11-800).
2. Many of our customers have been using syringes manually or a peristaltic pump to perfuse rodents. Though a gravity system may take longer, the results are reproducible and perfusion is thorough. Gravity systems allow consistent pressure and controlled flow rates, providing good perfusion of the major organs. Excessive pressure using other methods may cause artifacts in the brain histology.
3. Mice should take 10 to 20 minutes to perfuse thoroughly, requiring 10-25 ml of saline and 50-100 ml of fixative. Rats should take 10 to 30 minutes for 50 to 100 ml of buffer and 400-600 ml fixative.
4. Fixation is indicated when the animal exhibits vigorous muscle contractions and becomes rigid.
5. The In Vivo system is normally used to perfuse one rodent at a time, but has the capability to perfuse two simultaneously. AutoMate Scientific offers a 4-syringe clamp and the associated hardware to perfuse two at once. Contact us at info@autom8.com for more details.
6. We have included two different needles for you to try. The mouse system (AutoMate #11-140) has one 20 gauge straight needle and one 22 gauge butterfly needle. The rat version (AutoMate #11-800) has one 18 gauge straight needle and one 20 gauge butterfly needle. Feel free to order more needles through our web site: www.autom8.com.

References

There are many reliable sources for the procedure to perfuse rodents. Before proceeding, we recommend reviewing the following published sources:

- Flecknell, P.A., Chapter 2, Anesthesia and Perioperative care, Methods in Enzymology. In, *Guide to Techniques in Mouse Development,* Vol. 225, (Edited by Paul Wassarman & Melvin L. dePamphilis) p.16.
- Hockfield, S., Carson, S., et al., Chapter 3, Fixation by Transcardiac Perfusion. In, *Selected Methods for Antibody and Nucleic Acid Probes*, Vol. 1, pp. 125-130.
- Krinkle, Georg J. (ed.), Perfusion Fixation. In, *The Laboratory Rat*, pp. 518-521.
- Waynforth, H.B. and Flecknell, P.A., *Experimental and Surgical Technique in the Rat*, Second Edition, pp. 316-322.

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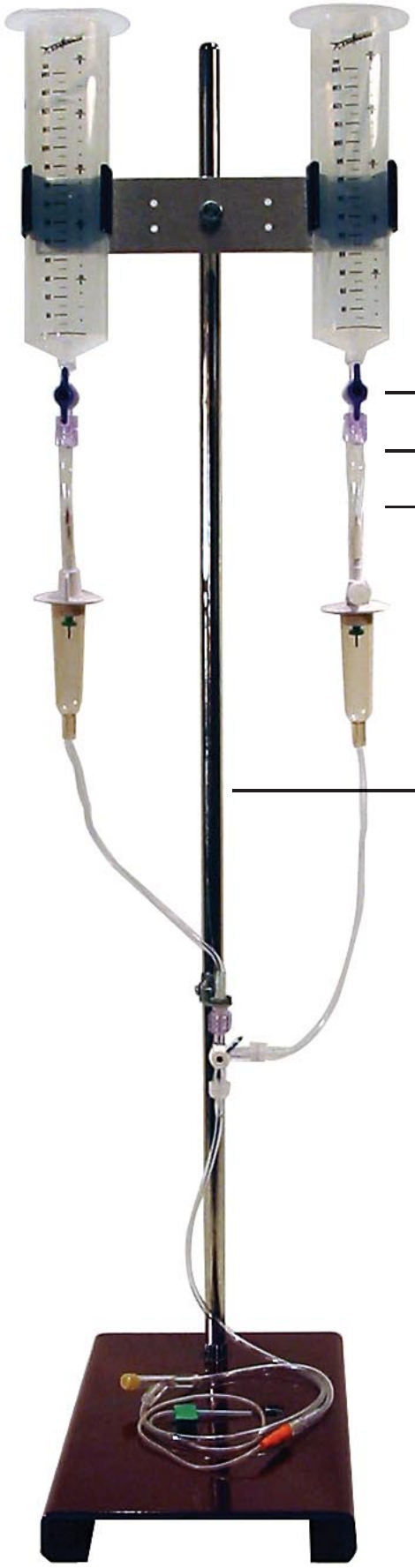

140ml syringe reservoirs (2 shown)
or 800ml beakers

Syringe bracket with Gripper clips
or 800ml reservoir bracket

Stopcocks (2)

Lure-lock hose barb fittings (2)

2" pieces of $^{3}/_{16}$" tubing (2)

Spiked drippers (2)

Ring Stand Pole

Dripper tubing

Ring Stand Clamp Assembly
with 3-way valve

Lower tubing
(left over dripper tubing)

Needle

Ring Stand Base

Diagram A

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Assembly Instructions

1. Take the components out of the box, ensuring that all components are accounted for (see diagram A).
2. Insert Ring Stand Pole into Ring Stand Base (either threaded hole).
3. Slide Ring Stand Clamp Assembly down Ring Stand Pole, until it is about 6" above the Ring Stand Base. The valve should be oriented below the Pole Clamp.
4. Using a flat-head screwdriver, tighten the screw until the clamp is secure on the pole (see Diagram B). Please note how the three-way valve operates (see Figure 1). One fluid enters the valve from the right, one from above, with the outlet draining out the bottom. To shut off all liquid flow, position the valve handle between the two entering liquids, at the 2 o'clock position. To allow fluid to flow from the reservoir on the right, position the valve handle at noon; the fluid from the left reservoir flows with the valve handle at 3 o'clock. ow

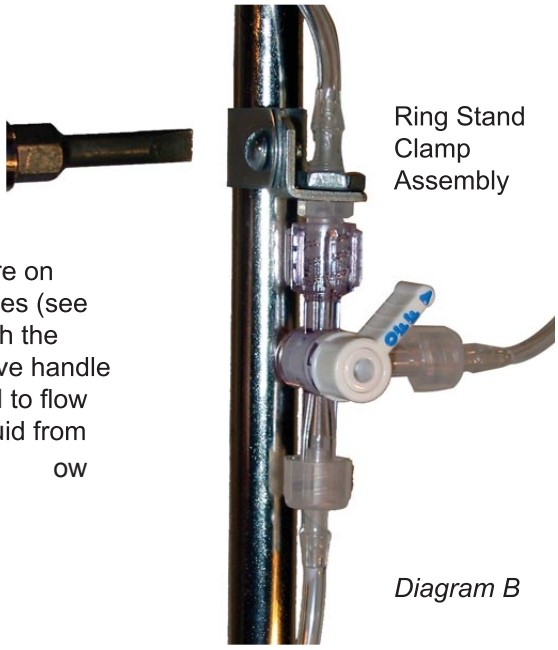

Diagram B

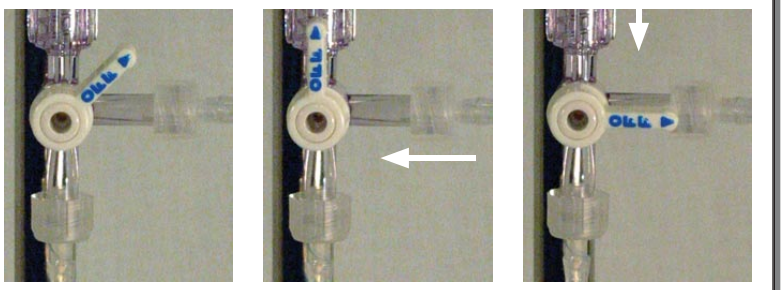

Figure 1

5. To maximize perfusion pressure, attach the reservoir holders to the top of the Ring Stand Pole. (For the Mouse version, use the Syringe Plate with Gripper Clips; for the Rat version use the Reservoir Bracket.)
6. Mouse system: Attach the syringes to the syringe clamps. Rat system: Insert the 800ml beakers into the Reservoir Bracket.
7. With a razor or scissors cut in half the approximately 4" piece of $^{3}/_{16}$" Tygon tubing. Each piece of tube then attaches to the hose barb fitting at the bottom of each reservoir and the spike at the top of each Dripper (see Figure 2). In order to facilitate this process, fill a coffee cup with hot water. By holding the last 1" of tubing in the hot water, you can soften the tubing and easily push it onto the hose barb (see Figure 3).
8. The next step is measuring and cutting the Dripper Tubes. At this point both Drippers should be attached to the reservoirs above them. Measure the length from the left Dripper (attached to the saline reservoir) down to the top of the 3-way valve. After adding an extra inch or two to the length of the tubing (to avoid tubing tension) cut the tubing with a razor or scissors and attach to the hose barb at the top of the 3-way valve. Ensure a secure fit by pushing the warmed tubing over the hose barb entirely (see Figure 3). Do the same with the right Dripper Tubing, allowing enough slack to attach to the right side hose barb on the 3-way valve (you can always cut it shorter). When the tubing cools and shrinks it should be difficult to pull off the hose barb.
9. At this point there should be two pieces of tubing remaining from the sectioned Dripper Tubes. Take one of them and measure the appropriate length of tubing desired to go from the bottom of the 3-way valve to the needle. Ideally the animals will be perfused right next to the Ring Stand, in which case the length of the tubing may only need to be 14"-18" long. Cut the tubing to the appropriate length, and attach to the hose barb.
10. Attach your needle to the luer lock fitting on the free end of the vinyl tubing.

Figure 2

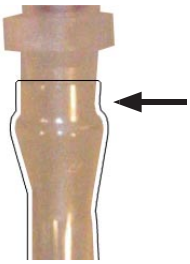

Figure 3

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Perfusion Technique

1. Put on appropriate safety gloves, glasses and labcoat. Remember that formaldehyde and paraformaldehyde are toxic – please follow MSDA guidelines.
2. Put the ring stand under the fume hood to avoid inhalation of fixative fumes
3. Fill one reservoir with saline and the other with fixative. Squeeze the drippers to help start flow. Drippers must be filled to their "fill line" to avoid air bubbles. Allow "saline" and "fixative" to run through tubing to remove any air bubbles. Drain into the fume hood sink. Finally, switch back to "saline" and let flow again, to purge any remaining "fixative" from the lower tubing (between the 3-way valve and needle).

> The smallest amount of residual fixative will arrest the proper flushing of blood, so be sure all fixative has been flushed from the lower tubing before starting!
>
> Air bubbles must be cleared before starting! They disrupt liquid flow, and will block capillaries.

4. Place the deeply anesthetized animal on its back on Styrofoam or paraffin block in a large container, all in the fume hood.
5. Spread the forelimbs and pin each paw to the Styrofoam or paraffin block with pins or syringe needles.
6. Surface sterilize the chest of the mouse with 70% ethanol. This step is optional, but also flattens the fur.
7. Grasp the end of the sternum with a forceps and with sharp scissors cut the skin, then diaphragm laterally on both sides and then cut upward across the ribs and parallel to the lungs exposing the heart.
8. While saline is flowing (optional), insert the needle into the left ventricle and as the heart fills up cut the right aorta and allow the blood to flow out until it is pale red. Allow approximately 10-25 ml of saline to wash through the mouse, 50-100 ml for a rat.
9. Turn the three-way valve mounted on the ringstand rod to the 12 o'clock position, switching to the fixative. Allow 50-100 ml (mouse) or 400-600ml (rat) of fixative to flow through the animal.
10. Complete the fixation of the animal, looking for vigorous muscle contractions to indicate fixation has occurred. Avoid liquid leaking out the nose of the animal since this is an indication that fixative is not going through the systemic circulatory system but rather back-filling fixative into the lungs and out the nose. This can be avoided this by advancing the needle up the apex of the left ventricle into the base of the aorta.
11. Once perfusion is complete, turn off the fixative (move valve to the 2 o'clock position). Remove the needle from the animal and put the tubing in the sink.
12. Set aside the animal, and empty the tray.
13. If perfusing more animals, return to step 3.
14. When finished, open the valve and drain remaining liquids from both syringes. Fill each syringe with water and drain. Repeat, to ensure all saline and fixative have been eliminated.
15. Rinse off the syringes and tubing. Wipe down the equipment with disposable towels (in the event there is fixative on equipment surfaces) before taking off gloves.
16. Hang dry the tubing.

Other Useful Information

Head pressure

- Flow rate and pressure are proportional to the height of the reservoirs. Raise them as high as possible on the ring stand pole provided for maximum flow rate.

Anesthetics

- Follow your institute Committee on Animal Research guidelines for using anesthetics
- Options include Avertin, Sodium Pentobarbitol or Metofane (methoxyflurane) inhalation—see reference above for dose and vendor recommendations

Perfusion Wash

- 1 x Saline (10x = 85g NaCL per liter in water)
- 1 x Phosphate buffered saline (PBS) (10x PBS = 74g NaCl, 9.94g Na_2HPO_4 , 2.4g Na H_2PO_4)
- Anticoagulants, such as heparin (1000 units/L), can be included in the wash buffer to improve perfusion.

Perfusion Fixatives

- 10% formalin or fresh 4% paraformaldehyde (PFA) formaldehyde.
- For electron microscopy check with your protocol for the best fixatives and time for fixation. Perfusion time, and therefore fixation times are critical for electron microscopy.

To make 4% PFA

1. 100 ml PBS - heat to 65˚ in microwave
2. Add 4g PFA and 100 µl 10 N Sodium hydroxide
3. Stir on magnetic stirrer in fume hood until dissolved. Filter sterilize and cool on ice.

For research use only.

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