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Mouse Perfusions with BriteVu®

1. Give 100 U (all sized mice) heparin (IV, IP or IM) 30 minutes prior to flushing blood.

2. Anesthetize the animal as per your IACUC, or other regulatory research, protocol.

3. Place an appropriately sized butterfly catheter or needle in the left ventricle.
   A. Place the mouse on an absorbent pad overlying a Styrofoam backing (pad).
   B. Surgically expose the ventral body wall at the thoracic-abdominal junction being careful to not cut the underlying liver.
   C. The diaphragm and lateral sides of the rib cage are carefully cut to expose the heart. Use a hemostat or needles (insect pins) to keep the rib cage peeled back away from the heart. Pins can be pushed through the rib cage and into the underlying Styrofoam backing.
   D. Direct a butterfly catheter or other needle into the left ventricle chamber. Common needle sizes for mice include 21-23 gauge. Carefully place the needle such that the heart muscle is not lacerated.
   E. Stabilize the needle. If using a butterfly catheter, pin the wings of the catheter (through pre-drilled holes) to the underlying Styrofoam backing. If using a needle, use a stabilization device such as spring-clamp workholders.
   F. Attach a flush filled IV extension line to the needle or use the line end already attached to the butterfly catheter. Make sure there are no bubbles/air pockets in the extension line. When flushing, do not put any pressure or apply movement to the catheter end. Use the free end of the extension line to attach syringes for flush and BriteVu solution perfusion.

4. Use warmed fluids to flush the blood from the vascular system.
   A. Make a precise incision into the right auricle. Make the incision less than 4 mm long. Larger incisions may significantly reduce systemic pressure and prevent a thorough vascular flush and perfusion.
   B. Use warm (37-39°C [99-102°F]) physiologic solution (0.9% NaCl, PBSS, etc) as vascular flush.
   C. Alternatively, 1% Dawn Ultra dish soap can be added to aid flushing. Add 10 cc Dawn Ultra to 1 L of flush solution.
   D. Use 200-400% of body weight in flush. Use 40-80 cc (ml) per 20 g of mouse body weight. Larger mice may need a smaller volume of flush.
   E. Use a syringe and determine ideal flush pressure using your hand. A syringe pump can be used. However, there is a risk of vascular rupture if a constant pressure pump is used and not adjusted during the perfusion.
   F. Blood and flush solution should be noted exiting the cut vessel. Excessive delivery pressure and/or speed may result in vessel rupture. As the flush progresses, the exiting fluid should turn from (blood) red to light pink.

5. Prepare BriteVu solution.
   1. Consider plain (distilled water), added 1-2% Dawn Ultra, Phenol, Alcohol or other preparations. See 'Protocols' for more details.

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Keep in a cool dry place. Keep out of the reach of children. Avoid contact with eyes and inhalation
In case of accidental exposure call 1-800-535-5053 (North America) or 1-352-323-3500 (International).

2. Plan for 200-400% of body weight in BriteVu solution. Use 40-80 cc (ml) per 20 g of mouse body weight. Larger mice may need a smaller volume of BriteVu solution.

6. Perfuse subject with BriteVu Solution.
   A. As with the flush solution, deliver the BriteVu perfusion via hand perfusion. A syringe pump can be used. However, there is a risk of vascular rupture if a constant pressure pump is used and not adjusted during the perfusion. If delivered too fast or with too much pressure (via hand or syringe) there is a risk of micro or large vascular rupture.
   B. BriteVu should be seen exiting the distant cut vessel after 10-25% of the calculated BriteVu is delivered. The lung vasculature will noticeably fill with BriteVu solution first.
   C. Make a window in the lateral thorax such that draining blood and BriteVu can drain away from the body. You may need to slightly angle the body and tilt the underlying Styrofoam backing to aid drainage away from the body.
   D. Monitor the tongue, eyes and peripheral vessels for evidence of adequate perfusion.

7. Once the perfusion is complete, cap off catheter or needle and leave in the heart.
   A. Place the subject in ice water to speed solidification and stop heat related tissue damage.
   B. Clean the skin and hair of any leaked BriteVu solution. BriteVu solution can simply be washed off with warm water, wiped or picked off. Once cooled, remove the needle/catheter and excess BriteVu (inside and outside the body).

8. Once the BriteVu has solidified, the subject is ready for scanning or storage in fixative.
   A. Harvested tissues, regions or whole body can be stored in formalin indefinitely and scanned later.
   B. Tissues can also be stored in various fixatives for histology, electron microscopy, etc.