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## Rat Perfusions with BriteVu®

1. Give 1000 U/Kg of rat body weight 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. **Perfusion set up method #1.**
  - A. Place the rat on an absorbent pad overlying a Styrofoam backing.
  - 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 rats 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 or 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. Place an appropriately sized IV catheter in the lateral saphenous vein. **Perfusion set up method # 2.**
  - A. Shave the lateral and caudal aspect of the rear limb just proximal to the hock (tibiotarsal joint).
  - B. Use alcohol to wet the skin and better expose the lateral saphenous vein.
  - C. Place a small tourniquet or use fingers to help make the saphenous vein distend.
  - D. Using a needle, blade or fine scissors, cut the skin adjacent to the vein at its lateral most aspect of the distal leg. Be careful to not lacerate the underlying vein.
  - E. Carefully place a catheter into the vein. Depending on the size of rat, 22, 24 or 26-gauge catheters generally work well. Use the largest catheter that will comfortably fit.
  - F. Secure the catheter in place with butterfly tape adhered to the catheter hub and sutured to the skin or any other catheter stabilizing tape procedures. If planning for whole body scanning, use radiolucent tape (such as 3M™ Transpore™ Surgical Tape).
  - G. Attach a flush filled IV extension line to the 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® mix perfusion.
5. Use warmed fluids to flush the blood from the vascular system.

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- A. Make an incision over a distal limb vessel away from the site of perfusion. If placing a lateral saphenous vein cut the tail or opposite leg. For left ventricular perfusion cut either rear leg or tail. Multiple limbs may need to be cut in some situations. Make a deep enough cut such that blood free freely flows.
  - B. Use warm (37-39°C [99-102°F]) physiologic solution (0.9% NaCl, PBSS, etc) as vascular flush.
  - C. As an alternative, use distilled water. Of note, distilled water commonly causes muscle fasciculations and uncoordinated body movements.
  - D. Use 30-40% of body weight in flush. Use 300-400 cc (ml) per 1000 g of rat body weight. Larger rats 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. Complete vascular flushing may take 4-7 minutes for most whole rats.
6. Prepare BriteVu® mix.
1. Consider plain (distilled water), added 1-2% BriteVu® Enhancer, Phenol or other preparations. See [‘Protocols’](#) for more details. The specific protocol will also determine perfusion temperature.
  2. Plan for 20-40% of body weight in BriteVu® mix. Use 200-400 cc (ml) per 1000 g of rat body weight. Larger rats may need a smaller volume of BriteVu® mix.
7. Perfuse subject with BriteVu® mix.
- A. As with the flush solution, deliver the BriteVu® mix 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® mix should be seen exiting the distant cut vessel after 10-25% of the calculated BriteVu® mix is delivered. The lung vasculature will noticeably fill with BriteVu® mix first.
  - C. You may need to slightly angle the body and tilt the underlying Styrofoam backing to aid drainage away from the body. Complete BriteVu® mix perfusion commonly takes 4-7 minutes for most whole rats.
  - D. Monitor the tongue, eyes and peripheral vessels for evidence of adequate perfusion.
8. Once the perfusion is complete, cap off catheter or needle and leave in the heart, rear leg or other location.
- 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® mix. BriteVu® mix can simply be washed off with warm water, wiped or picked off. Once cooled, remove the needle/catheter and excess BriteVu® mix (inside and outside the body).
9. Once the BriteVu® mix 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.

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