CRYOEMBEDDING

Supplies:

Ice bucket
Dry Ice (stored in –20° C walk-in freezer)
2-methyl butane (stored in acid cabinet)
Aluminum foil
Small glass vial
Weigh boats
Forceps
OCT (Optimal Cutting Temperature, Electron Microscopy Sciences)
Alcohol-resistant marker (very important)

Procedure: Fixed Tissue

1. Following fixation (immersion or perfusion), rinse tissue in phosphate buffer (PB, 0.1M, pH 7.3) containing 5, 10, and 20% sucrose for 24 hours per step or until the tissue "sinks" to the bottom of the container or tube.

The final sucrose concentration in the tissue may vary from 5-30% depending on what conditions work best for the tissue in question.

2. Cool 2 methyl-butane in a dry ice bath until dry ice no longer boils when added.
3. Make embedding molds out of aluminum foil. Cut small squares, about 1" square or less. Use the bottom of a small glass vial or appropriately sized mold, form foil squares around bottom.
4. While foil is still on the mold, write all information concerning the tissue going in the mold.
5. Remove foil from mold, add OCT.
6. Take tissue out of 20% sucrose and place in a small dish or weigh boat containing OCT to remove any excess sucrose.
7. Place tissue in foil mold. Make sure all tissue is flat on the bottom of the mold. Cover tissue with OCT.
8. Using forceps, hold mold in 2-methyl butane until OCT freezes. Try not to drop the mold in the 2-methyl butane, as the block will freeze too fast and crack.
9. Keep in bucket on dry ice until all blocks are ready to be put in –20° C or –80° C freezer.Fold excess foil over the top of the block to prevent freeze-drying.

Procedure: Fresh Tissue

Blocks of fresh tissue may be snap frozen at approximately -40° C and immediately sectioned on the cryostat or covered in OCT, snap frozen and stored at for later sectioning. The freezing temperature is critical, the slower the tissue freezes, the more ice crystals form. If the piece of tissue is very large there is a risk of it shattering if frozen too fast.