

## Fresh Frozen Tissue Preparation Protocol

### **Material preparation:**

1. Tissue-Tek O.C.T. compound (Sakura Finetek from VWR, cat#25608-930).
2. Plastic mold (choosing proper size of mold depend on the specimen).
3. 2-methylbutane (Fisher Scientific, cat#O3551-4).
4. Dry ice.

### **Procedure:**

1. Preparing cold 2-methylbutane bath.  
Pour 2-methylbutane in a metal bowl or plastic beaker. Add small pieces of dry ice into the solution to cool the solution down to  $-40^{\circ}\text{C}$  to  $-50^{\circ}\text{C}$ .
2. Labeling mold.
3. Placing tissue on a paper towel to get rid of excess liquid before you put the tissue into the O.C.T.,
4. Choosing proper orientation of the tissue depend on your interest (tissue will be cut starting from the bottom of the mold).
5. Adding one drop of OCT to the bottom of the mold; put tissue on the top of it; then add more OCT to cover the tissue and the whole mold.
6. Hold the mold with hemostat and let bottom of the mold touch the cold 2-methylbutane.
7. Dropping the mold into cold 2-methylbutane when it is completely white and let it stay in cold bath for another 5 minutes. Take the tissue out and keep it on dry ice.
8. Repeat the process if you have more than one tissue to freeze.
9. Wrap frozen blocks with labeled foil and store them at  $-80^{\circ}\text{C}$  freezer.

### **Procedure Note:**

1. Dissect the tissues as quick as you can to maintain the best morphology.
2. Do not put tissue in any liquid.
3. Keep adding dry ice to the cold bath to keep the temperature low.
4. Choose appropriate size of frozen mold for your tissues.
5. Keep in mind that temperature fluctuation can damage tissue's integrity.