

## **Tissue Prep for SDH & ATPase Stain**

**Tragacanth** (prepare at least one day before sample harvest)

5 g Gum Tragacanth (Sigma G1128)  
50 ml Millipore H<sub>2</sub>O  
1 ml phenol

Add ingredients in 50 ml conical, mix, and let sit in fridge O/N to soak

### **Isopentane Freezedown**

- Cool down isopentane in metal beaker that is immersed in liquid N<sub>2</sub> (until isopentane starts to freeze)
- Prepare Embedding Blocks (Fisher Scientific No. 6755810):  
Use spatula to sculpture Tragacanth pyramid on top of grid  
Form cavity for the sample at top of pyramid
- Mount muscle sample on top of Tragacanth pyramid (longitudinal, so that you will get cross sections)
- Cover whole muscle with Tissue-Tek O.C.T. compound (Sakura No. 4583)
- Use big forceps to immerse block w/ tissue on top into isopentane for ca. 10 sec.
- Transfer block w/ sample to -80 °C right away (tissue must not thaw once it is frozen since this will lead to destruction; if you put sample in liquid N<sub>2</sub>, it will crack)
- If Tragacanth cracks (that happens), apply O.C.T. compound on cracks and put in -80 °C before the tissue thaws

### **Cutting Samples in Cryotome**

- Transfer samples to Cryotome on dry ice
- Adjust cryotome: -20 °C; 10 Micron slices
- Let samples adjust to -20 °C in cryotome for 15 min
- Mount embedding block with tissue in cryotome
- Unlock advance mechanism
- Advance or retract sample so that is close to touching the knife
- Lock advance mechanism
- Use wheel on right side of cryotome to advance sample repeated times until the O.C.T. compound on top of the sample is cut off
- Flip down plexiglass shield on the blade
- Cut 2 pieces of sample
- Pick up sample with microscope slide (Fisherbrand Superfrost/Plus, No. 12-550-15)
- Wipe off blade (if you picked up sample properly, there should be only a weak “phantom” remaining that comes off easily)
- Cut more!