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Processing Protocol for Frozen Tissues

Materials

- OCT compound (Available at Central Stores, Tupper Bldg.)
- Sucrose
- EM capsules
- Wooden racks (available at Histology, 11G1, Tupper Bldg.)
- Liquid nitrogen
- Dipping holder for capsules (available at Histology, 11G1, Tupper Bldg.)
- Ziploc bags

Procedure

- Prepare embedding medium by mixing 2 parts OCT: 1 part 20% Sucrose in PBS and store at 4°C.
- Label capsules on the top with one letter or number using a black marker.
- Fill capsules half way with OCT-sucrose and place dissected tissue at the bottom of the conical shaped capsule (this will be the area where sections will be taken). Try to orient area of interest towards this end.
- Fill capsules until tissue is completely submerged; use a blunt needle or Pasteur Pipette to push tissue down until medium infiltration begins. Leave at room temperature 15-30 minutes.
- Place capsule in dipping holder and quickly freeze in liquid nitrogen for 10 seconds.
- Put frozen blocks in a Ziploc bag; include a legend with proper tissue identification and keep a photocopy for your freezer inventory records.
- Store at -70°C to -80°C.
- After cutting, wrap remaining tissue block in tin foil. Put back in Ziploc bag and store in the -70°C freezer for further use.