

Cryo EM tissue preparation.

1. Fix tissue mildly. Trim into small (<1mmsq) blocks.
2. Infuse tissue with a cryoprotectant for at least one hour (PVP/ sucrose).
3. Mount onto cryo pins, and freeze rapidly in liquid nitrogen.
4. Store pins in cryo vials in liquid nitrogen dewar.

Immunolabeling Experimental protocol.

1. Cut ultra thin sections (50-80nm)
 2. Pick up section with sucrose drop.
 3. Place on formvar / carbon coated Nickel grid.
 4. Wash off excess sucrose.
 5. Block for nonspecific binding with 10% FCS 1 hour.
 6. Place grid section side down on 10ul drop of diluted antibody. for several hours / overnight.
 7. Wash with blocking buffer.
 8. Incubate grids section side down on secondary antibody, (5-10nm IgG colloidal gold).
 9. Wash blocking buffer/ wash water.
 10. Stain 20 mins with neutral UA.
 11. Wash with water.
 12. Stain and embed section in a monolayer on PVA / Methyl cellulose.
- Let dry and scope.

Protocol taken from the W.M. Keck Microscopy Facility at The Whitehead Institute website