

IMMUNOCYTOCHEMICAL PREPS

Fixation of Tissue

1. Fix tissues/cells for only one hour in cryofix.
2. Spin down in small table top centrifuge at about 850 x g.
3. Resuspend cells in 3% liquid gelatin. Place on ice for 10 min.
4. When gelatin is solid, add more cryofix.
5. After 15 minutes, loosen pellet from the sides of the tube.
6. Fix for 15 more minutes.
7. Place in 2.3 M sucrose with PVP solution.
8. Leave overnight in fridge.

Freezing of Tissue for Cryo Storage

9. Collect 2 pairs of forceps, cryo storage tubes, clean screws and liquid N₂.
10. Label the cryotubes with EM #, date, PI, and specific sample #.
11. **Bore holes into the top half of the tube with a needle** and place the tube in liquid N₂.
12. Pour sucrose/tissue mixture into petri dish.
13. Mince pellet into 0.5 mm cubes.
14. Freeze the blocks one at a time, one block per screw, by immersion in liquid N₂.
 - Place the screw in the cryo tube immersed in liquid N₂.
 - It is now very important that the tissue remain frozen.
15. Store in R2D2.

Solutions List:

- 1 x PBS [Phosphate Buffered Saline, 10X Solution, Fisher Scientific]
- 2.5% Glutaraldehyde in PBS [25% Taab Glutaraldehyde, EM Grade, Energy Beam Sciences]
- Cryofix Fixative(2% Paraformaldehyde; 0.01% glutaraldehyde in PBS)[Formaldehyde, Para, Certified, Fisher Scientific]
- 2.3 M Sucrose in PBS [Sucrose, Fisher Scientific]
- 20% PVP 1.6 M Sucrose buffered with 0.055 M Sodium Carbonate [polyvinylpyrrolidone and Sodium Carbonate, Fisher Scientific]
- 1% Osmium Tetroxide (OsO₄) in dH₂O [Osmium Tetroxide, Electron Microscopy Sciences]
- 1% Osmium Tetroxide (OsO₄) + 1 % Potassium Ferricyanide [Potassium Ferricyanide, Fisher Scientific]
- Ethanol (30% in PBS, 50% in PBS, 70% in dH₂O, 95% and 100%) [(30- 95%) Reagent Alcohol, Fisher Scientific, and 100% requisitioned in pints]
- Propylene Oxide [Propylene Oxide, Electron Microscopy Sciences]
- Epon Resin [Scipoxy 812, DDSA, NMA, and DMP-30, Energy Beam Sciences]
- LR White Resin [LR White Resin, Electron Microscopy Sciences]
- 0.5% Toluidine Blue in 1% Sodium Borate [Toluidine Blue O Certified Biological Stain and Sodium Borate, Fisher Scientific]
- Sodium Hydroxide [Sodium hydroxide pellets, Fisher Scientific]
- Reynold's Lead Citrate [Lead Nitrate and Sodium Citrate Dihydrate, Fisher Scientific]

- 4% Uranyl Acetate [Uranyl Acetate, Electron Microscopy Sciences]