**Tissue/Cultured Cells - Membrane Preservation**



Procedure for fixing and embedding animal tissue and/or cultured cells to preserve membranes morphology

**Protective Equipment**; Safety glasses, Lab coat, gloves and Chemical Fume hood

Remember to **label all vials** with; sample name, fixative type, your name & date

Fixation: 

4%Paraformaldehyde/4% Glutaraldehyde in 0.1M Cacodylate buffer 10 mins at room

2% Paraformaldehyde/2.5% Glutaraldehyde in 0.1 M Cacodylate buffer w/0.2 M sucrose 2 hr @ rt

Rinse: 0.1M Cacodylate buffer w/0.2 M sucrose 3x 5 min

Post Fix: 2% OsO4 in 0.1M Cacodylate buffer 2 hr, rt

Rinse: ddH2O 3x 5 min

Stain: 1% Tannic Acid (not hazardous) 1 hr, rt

Rinse: ddH2O 2x 5 min

Optional step:  1 - 2% Uranyl Actate 2 hrs, rt

Rinse: ddH2O 10 min

Dehydration:  30% Ethanol 10 mins

50% Ethanol 10 mins

70% Ethanol 10 mins

95% Ethanol 10 mins

100% Ethanol 3x, 10 m ea

Transfer from plastic container to scintillation vials

Infiltration: 100% Acetone 2x 10 mins

1:1 Acetone: SPURRS  1 hr at rt

100% SPURRS open vial 2 hr at rt

Embed in SPURRS 70° C 16 hr

Reference:

N. Simionescu and M. Simionescu, J. Cell Biology (1976-70), 608-621

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