Cell Culture Fixation/Embedding - Epon

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

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

Grow cells in well plate on Thermanox cover slips, observe sterile techniques.

Wash 3x in buffer \*

Fixation: 2.5% Glutaraldehyde in 0.1 M buffer 1 h in well



Rinse: With buffer\* at the same concentration as fixative buffer 3x 10 mins each

Optional: Osmium tetroxide (1-2%) 1h room temp (r.t.),

 or overnight 4° C

Rinse: With ddH2O after OsO4 3x 5 -10 mins each

Dehydration: Before dhydrating, move cover slip carefully to scintillation vial

 30% Ethanol 10 m

 50% Ethanol 10 m

 70% Ethanol 10 m

 95% Ethanol 10 m

 100% Ethanol 10 m each

 Ethanol : Acetone

 1:1 10 mins

 100% Acetone 2x, 10 mins ea

Embedding: Acetone : Epon Mixture

 2 : 1 1 h

 1 : 1 1 h

 100% 2x, 1h

 Fill mold with Epon and place cover slip cell-side down on resin, with edges of cover slip hanging over the edges of the mold.

Curing: 60º C overnight

Cover slip removal: Snap sample out of mold. Place sample, cover slip side down, on very hot hot plate. Peel sample off cover slip. If sample does not come off easily reheat.

\* 0.1 M Phosphate buffer, PBS or Cacodylate

