**Cell on substrate**

Conventional glutaraldehyde fixation for TEM of animal cells in culture

**Fix preparation**

* 10 ml 25% glutaraldehyde
* 50 ml buffer stock solution (ie 0.2 M sodium cacodylate buffer, with 4 mM CaCl2, pH 7.3-7.4)
* Make up to 100 mls with distilled water
* Adjust pH with HCl to pH 7.3-7.4
* Final concentration: 2.5% glutaraldehyde in 0.1 M sodium cacodylate with 2 mM CaCl2.
* **Always** keep glutaraldehyde in the fume hood. Wear gloves: sodium cacodylate contains arsenic, glutaraldehyde fixes skin.

**Wash buffer preparation**

* 1:1 Stock buffer with distilled water
* Stock buffer: 0.2 M Stock sodium cacodylate buffer, with 4 mM CaCl2
  + 4.28g in 100 mls ddw = 0.2 M
  + 0.044g CaCl2 in 100 mls = 4 mM
  + adjust pH with HCl to pH 7.3-7.4
  + Final concentration: 0.1 M sodium cacodylate with 2 mM CaCl2, pH 7.3-7.4.

**Fixation**

1. Warm fixative to temperature at which cells are growing.
2. Pellet cells in Ependorf tube.
3. Remove medium.
4. Add fix.
5. Resuspend.
6. Fix for 1/2-1 hour.

**Wash in buffer**

1. Wash in 0.1 M sodium cacodylate buffer for 3 x 5 minutes.

**Post fixation:**

1. 1:1 Osmium 2% stock with 0.2 M cacodylate buffer stock.
2. Final concentration 1% osmium tetroxide, 0.1 M cacodylate buffer.
3. 20-30 minutes.
4. Add just enough osmium to cover cell pellet.

**Rinse**

1. Fill Eppendorf with distilled water.
2. Turn upside down so any osmium in the lid is removed.

**Enbloc stain**

1. 2% Uranyl acetate for 30 minutes.

**Dehydration**

1. Empty rinse water and fill immediately with 30% alcohol.
2. Turn vial upside down once. This is to ensure no water remains in the lid to contaminate/rehydrate the sample.
3. Put vial on rotater for 5 minutes.
4. Empty 30% and replace immediately with 50%. Turn upside down once.
5. Repeat with 70%, 85%, 95%, 100%, 100%. 5 minutes in each alcohol.

**Embed, for cultured cells**

1. 3:1 ethanol:Spurr resin for 30 minutes
2. 1:1 ethanol:resin for 30 minutes
3. 1:3 ethanol:resin for 30 minutes
4. 100% resin 1 hour.
5. If the pellet is particularly large, leave overnight in fresh resin.

**Polymerise resin**

1. Fresh resin, in 60 C oven overnight (16 hrs).