**Animal tissue**

Conventional glutaraldehyde fixation

**Fixative 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.

**Washing buffer preparation**

* 1:1 Stock buffer with distilled water
* Final concentration: 0.1 M sodium cacodylate with 2 mM CaCl2, pH 7.3-7.4.
* 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

**Fixation**

1. Cut samples into small blocks (1 mm cubes) with the sample immersed in fixative. Use a grease free, sharp razor blade or equivalent.
2. With a toothpick or small applicator stick, pick up a dozen or so small (1 mm3) blocks of tissue and transfer them to the vial of fixative (5-7 ml).
3. Fix on rotator at room temperature all day (8 hours) (varies with thickness of tissue: cells may take only half an hour)

A word of caution: Be careful with fixatives; they are noxious; avoid contact with skin or vapors. They are damaging to the eyes and mucous membranes of the nose, throat and lungs. It is essential to work in the fume hood. This same precaution also applies to the embedding materials.

**Wash in buffer**

1. At the end of the day, remove the fixative and rinse the tissue blocks in 0.1M cacodylate buffer at pH 7.3-7.4.
2. Put into fresh buffer and leave overnight in the refrigerator.
3. Next morning, rinse in 0.1 M sodium cacodylate buffer.

Take care not to loose or damage the small tissue blocks. They must be handled gently and must not be allowed to dry at any time during transfer.

**Post fixation**

1. 1:1 Osmium 2% stock with 0.2 M cacodylate buffer stock.
2. Post-fix for one hour in 1% osmium tetroxide. OsO4 is very toxic; the vapors are extremely noxious, so be careful. Wear gloves and open in fume hood.
3. Final concentration 1% osmium tetroxide, 0.1 M cacodylate buffer.

**Rinse**

1. Remove the OsO4 and fill vial with distilled water.
2. Turn upside down once.

**Dehydration**

1. Empty rinse water and fill immediately with 70% 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 10 minutes.
4. Empty 70% and replace immediately with 85%. Turn upside down once.
5. Repeat with 95%, 100%, 100%, 10 minutes in each alcohol.

For most pieces of tissue you can start at 70% ethanol (10 minutes each). For cultured cells or cells with an abundance of vesicles start at 30% ethanol (5 minutes in each alcoholRepeat in 50%, 70%, 85%, 95%, 100%, 100%.

**Embed**

* + For large pieces of tissue
1. 100% propylene oxide for 30 minutes
2. 1:1 PO:resin for 1 hour
	* For normal pieces of tissue (1 mm cubes)
3. 1:1 EtOH:resin for 1 hour
4. 100% resin for one hour
5. 100% resin overnight

**Polymerise resin**

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