**Plant tissue**

Standard glutaraldehyde fixation

**Fixative preparation**

* 10 ml 8% glutaraldehyde
* 10 ml buffer stock solution (ie 0.2 M sodium cacodylate buffer, pH 6.8-7.2)
* Make up to 40 mls with distilled water
* Final concentration: 2.0% glutaraldehyde in 0.05 M sodium cacodylate.

**Washing buffer preparation**

* 1:3 Stock solution with distilled water
* Final concentration: 0.05 M sodium cacodylate

Always keep glutaraldehyde in the fume hood. Wear gloves: sodium cacodylate contains arsenic, glutaraldehyde fixes skin.

**Fixation**

1. Cut samples into small blocks with the sample immersed in fixative.
2. Fix for 8 hours (varies with thickness of tissue: cells may take only half an hour).

**Wash in buffer**

1. Rinse in 0.05 M sodium cacodylate buffer.
2. Wash overnight in buffer.
3. Rinse in buffer.

**Post fixation**

1. 1:1 Osmium 2% stock with 0.1 M cacodylate buffer stock.

Final concentration 1% osmium tetroxide, 0.05M cacodylate buffer.

**Rinse**

1. Fill vial with distilled water.
2. Turn upside down once.

**Dehydration**

1. Empty rinse water and fill immediately with 30% alcohol.
2. Turn vial upside down once.
3. Put vial on rotater for 20 minutes.
4. Empty 30% and replace immediately with 50%.
5. Turn upside down once. This is to ensure no water remains in the lid to contaminate/rehydrate the sample.
6. Repeat with 70%, 85%, 95%, 100%, 100%. 5 minutes in each alcohol.

**Embed**

1. 3:1 EtOH:Spurr resin for 0.5-1 hour
2. 1:1 EtOH:Spurr resin for 0.5-1 hour
3. 1:3 EtOH:Spurr resin for 0.5-1 hour
4. 100% resin for 1 hour

**Polymerise resin**

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