**Plant tissue**

Standard glutaraldehyde fixation

**Fix preparation**

1. 10 ml 8% glutaraldehyde
2. 10 ml buffer stock solution (ie 0.2 M sodium cacodylate buffer, pH 6.8-7.2)
3. Make up to 40 mls with distilled water

Final concentration: 2.0% glutaraldehyde in 0.05 M sodium cacodylate. Plasmolysis sets in with too high an osmolarity.

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

**Washing buffer preparation**

1. 1:3 Stock solution with distilled water
2. 0.2 M sodium cacodylate buffer, pH 6.8-7.2

Final concentration: 0.05 M sodium cacodylate.

**Fixation**

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

**Buffer wash**

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

**Post fixation**

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

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

**Rinse**

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

**Dehydration**

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

**Critical point dry run**