**Animal tissue**

Standard glutaraldehyde fixation

**Fixative preparation**

1. 10 ml 25% glutaralehyde
2. 50 ml stock buffer solution (i.e. 0.2 M sodium cacodylate buffer, pH 7.3-7.4)
3. Make up to 100 ml with distilled water

Final concentration: 2.5% glutaraldehyde in 0.1 M sodium cacodylate.

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

**Buffer preparation**

1. 1:1 Stock solution with distilled water
2. Stock: 0.2 M sodium cacodylate buffer, pH 7.3-7.4

Final concentration: 0.1 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 0.1 M sodium cacodylate buffer. Wash overnight in buffer. Rinse in buffer.

**Post fixation**

1. 1:1 Osmium 2% stock with 0.2 M cacodylate buffer stock.
   * Final concentration 1% osmium tetroxide, 0.1M cacodylate buffer. 1 hour

**Rinse**

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

**Dehydration**

1. Empty rinse water and fill immediately with 70% alcohol. Turn vial upside down once. This is to ensure no water remains in the lid to contaminate/rehydrate the sample. Put vial on rotater for 10 minutes.
2. Empty 70% and replace immediately with 85%. Turn upside down once.
3. 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 alcohol). 30% ethanol, 50%, 70%, 85%, 95%, 100%, 100%