

## Routine Preparation of Tissue for Transmission EM

Resin embedding:

All of these steps should be performed in the fume cupboard at room temperature unless otherwise stated.

1. Fix 1 mm<sup>3</sup> tissue pieces by immersion in 4% paraformaldehyde with 2.5% glutaraldehyde in 0.1M sodium cacodylate pH 7.4 (4 h-o/n)
2. 0.1M sodium cacodylate buffer wash pH 7.4 (5 min)
3. 1% osmium tetroxide in distilled water (1.5 h)
4. Uranyl acetate (2% uranyl acetate in 0.69% maleic acid or alcoholic 5% uranyl acetate) (3 x 30 min)
5. Dehydration: RT
  - 50% ethanol (2 x 5 min)
  - 70% ethanol (2 x 5 min)
  - 90% ethanol (2 x 5 min)
  - 100% ethanol (must be high purity) (3x 10 min)
  - 100% acetone (must be high purity) (3 x 5 min)
6. Resin infiltration:
  - 30% resin:70% acetone (1 h)
  - 70% resin:30% acetone (1 h)
  - 100% resin (2 x 1 h)
7. Place tissue in resin mould, pour in fresh resin and add label (use pencil)
8. Leave in oven to polymerise at 60°C overnight

Blocks of tissue are now ready to section

### **Notes:**

Fixatives and osmium tetroxide can be obtained as pre-made stock solutions.

0.2M sodium cacodylate buffer

WEAR GLOVES, DO NOT INHALE POWDER – ARSENICAL COMPOUND

10.7 g sodium cacodylate in 250 ml distilled H<sub>2</sub>O adjust pH to 7.4. Store at 4°C.

Uranyl acetate

WEAR GLOVES, DO NOT INHALE POWDER, CUMULATIVE TOXIN.

Dissolve 0.69 g maleic acid in 100 ml distilled H<sub>2</sub>O, then add 2 g uranyl acetate. Store at 4°C