Embedding with Spurr's Resin for EM

1. Fix tissue in 1% glutaraldehyde, 1 % paraformaldehyde in 0.86M sodium phosphate buffer, pH 7.3 overnight or 24 hours.

At 4^{o}C:

2. Rinse tissue in 0.137M sodium phosphate wash buffer, 3 Xs 15min.

3. Fix in 2% osmium tetroxide in 0.86M sodium phosphate buffer for 1 hour if the tissue is to be used for EM only (i.e. no immunocytochemistry). If post-embedding immunocytochemistry is to be done, fix in 0.5 % osmium for 20 min. (See protocol in Robert Marc's lab.)

4. Rinse in ddH2O for 15 min.

5. Dehydrate tissue in graded ethanol starting with 30%, 50%, 70%, 85%, and 95% for 10 minutes each. End with 3 rinses in 100% ethanol, 10 minutes each.

At 20^{o}C:

6. Rinse in propylene oxide, 3 Xs 20 minutes.

7. Infiltrate with propylene oxide / Spurr's (2:1) for 30 minutes.

8. Infiltrate with propylene oxide / Spurr's (1:2) for 30 minutes.

9. Infiltrate with 100% Spurr's resin, in capped vials, overnight on a rotator.

10. Embed tissue in fresh Spurr's resin and place in 65^{o} C oven and polymerize for 2 days (check for hardness after 24 hours).

Recipes:

A) Phosphate buffers:

1. Stock sodium phosphate buffer, 0.172M, pH 7.2. Add the following phosphates sequentially to the desired volume of ddH2O. Mix well with a magnetic stir bar.

A) To make 100 ml, add 0.68g NaH2PO4-H2O and 1.73g Na2HPO4 to 100 ml ddH2O.
B) To make 500 ml, add 3.41 NaH2PO4-H2O and 8.66 Na2HPO4 to 500 ml ddH2O.

C) To make 1000 ml, add 6.82 NaH2PO4-H2O and 17.32 Na2HPO4 to 1000 ml ddH2O.

2. Isotonic sodium phosphate wash buffer, 0.137M, pH 7.2.

To 4 parts stock buffer (0.172M) add 1 part dd H20. This will yield a sodium phosphate buffer of 0.137M pH 7.2, which is essentially iso-osmotic with mammalian blood (about 315 mOsM).

B) 1% Paraformaldehyde, 1% Glutaraldehyde in 0.086M phosphate buffer

1. To ≈ 450ml distilled H_20, add 10.0g paraformaldehyde.

2. Heat solution on a hot plate to almost 80^oC.

3. Clear with 5-10 drops of 1N NaOH. Let cool.

4. Add 500ml of 0.172M phosphate buffer stock, pH 7.2.

5. Add 14.3ml of 70% glutaraldehyde.

6. Bring final volume up to 1000ml.

7. Store in fridge at 4^oC.

C) 4% Osmium tetroxide stock solution: (purchased from Polysciences)

1. Using a hood and wearing gloves, break OsO_4 ampoules in a thick sided bottle. DANGEROUS OXIDIZER: avoid any contact!!

2. Add double distilled H_20, seal the lid of the bottle with parafilm and leave in the hood at room temperature for a day or two until crystals dissolve. Invert or swirl the bottle every few hours. Final solution will be a pale yellow.

3. Store in fridge at 4^oC. LABEL BOTTLE: CAUTION!!!

4. Over time the solution will start to turn from pale yellow to gray and ultimately to black. Only use when pale yellow.

5. Discard in properly labeled Toxic waste container.

For 25ml, dissolve 1.0g OsO_4 in 25ml dd H_20.

D) 2% OsO_4 in 0.086M phosphate buffer

Combine equal volumes of 4% OsO_4 stock with 0.172M phosphate buffer stock.

E. Spurr's Resin: (purchased from Polyscienes).

Each recipe makes about 45 ml.

E.R.L.	10.0g
D.E.R.	8.0g
N.S.A.	26.0g
D.M.A.E.	0.4g
Polycut EASE	0.45g

The values given in the table above represent weights of plastic and it is convenient to simply weigh out these materials on a top loading balance in a disposable beaker. When mixing the components add the accelerator, DMAE last, and only after the previous plastics had been stirred. The tissue can be embedded in any of a number of trays. For larger sample we use tins large enough to embed 1- 4 quadrants of an eye.