Fixation and embedding in LR White resin for light microscopy

FIXATION AND EMBEDDING MUST BE CARRIED OUT IN THE FUMEHOOD. WEAR GLOVES.

Materials

1. Buffer: 0.1M phosphate buffer pH 7.2 (stock)
2. Buffer: 0.05M phosphate buffer pH 7.2
3. Fixative: 4% paraformaldehyde+2.5% glutaraldehyde in 0.05M phosphate buffer
4. Ethanol
5. Glass vials
6. Paper/card to write labels in pencil and place inside vial
7. Plastic capsules for embedding – make sure they are the correct ones as LR white needs oxygen-free environment to polymerise.
8. Oven
9. Tweezers

Method

1. Prepare fixative in fume hood as follows:

For 100ml:

- Weigh 4g paraformaldehyde powder into a 30ml conical (Falcon) tube with a screw top, and add 30 ml dH_2O.
- Heat to approximately 70ºC (screw cap on loose) in a beaker of water on a hotplate. Check temperature regularly with a thermometer – do not let it boil.
- Once at 70ºC and the powder starts to dissolve, carefully add 1-4 drops of 1M NaOH using a Pasteur pipette until the solution becomes clear (a few white bits may remain undissolved) – *Do not ad too much NaOH as this will raise the pH too much!*
- Place the tube on ice and allow to cool to room temperature.
- Add Glutaraldehyde (10ml of a 25% solution) and make up to 50ml with distilled water.
- Mix equal volumes of glut/paraformaldehyde mix and 0.1M phosphate buffer. Check pH 7.2 (might need slight adjustment with NaOH). Keep at 4ºC until use.

2. Fix - 2h at room temperature (RT) with rotation and then at 4ºC overnight.

3. Wash - 3 x 0.05M phosphate buffer – 15-30min in each change. Samples can be stored at 4ºC in buffer for a few days.

4. Dehydrate in graded ethanol series (these times can be increased if needed depending on size of samples):

1. 10%EtOH rt 30 min
2. 20%EtOH rt 30min
3. 30%ETOH rt 30min
4. 40%EtOH rt 2x30min
5. 50%EtOH rt 2x30min
6. 60%EtOH rt 2x30min
7. **70%EtOH 4°C overnight**
8. 80% EtOH 2x30min
9. 90%EtOH 2x30min
10. 100% dry ETOH 3x20min

5. Infiltration in increasing concentration of resin.

- The times can be increased, especially if dealing with large and/or hard samples.
- A mild vacuum can help infiltration when samples are in pure resin.

1. Ethanol 100% : LRWhite 4:1 1hr
2. Ethanol 100% : LRWhite 3:2 1hr
3. Ethanol 100% : LRWhite 2:3 1hr
4. Ethanol 100% : LRWhite 1:4 1hr
5. 100% LRWhite 1hr then 4°C overnight.
6. 100% LRWhite 1hr at RT with rotation.

Note: the resin must be brought to room temperature before use. Only freshly opened resin should be used.

6. Embedding into capsules

- Label capsules and fill around ¼ of the capsule with resin.
- Place sample into capsule, ensure the sample is in the correct position. (If it is a challenging sample use a small amount of cotton wool to help hold it in position).
- Fill the rest of the capsule with resin and place in rack.

7. Nitrogen oven

- Check the taps are not clogged using the tweezers in the fume hood.
- Place samples in the oven with the lid on. Close the right hand tap and open the left.
- Turn on the nitrogen (blue tap on fume hood, turn 180^{o}). Leave this for 5-10 minutes.
- Open right hand tap. Leave for 5-10 minutes.
- Close both taps and turn off nitrogen.
- Leave samples in the oven for 16 – 20 hrs to polymerise.