Fixation and Embedding of Membrane Pellets

April 23, 1999

1. Spin isolated membrane fractions (e.g. 50-100 µl) in the SW55 at 40-50k rpm for ~30-60 min. Use the clear cellulose nitrate tubes.

1. While this is spinning, prepare fixative. To make up tannic acid (use good stuff), make up a 4% tannic acid in water. Tannic acid starts out being very acidic. Bring it up slowly with 1-4 N NaOH to neutral pH (between 7 and 8). Color will be yellow to start with and will turn more golden as the pH is raised. If you raise the pH too fast then the solution will turn brown and precipitate. If this happens, throw away the tannic acid and start again.

1. Fixative (final concentration) consists of:
   - ~1% tannic acid
   - 1.25 – 2% glutaradehyde
   - 0.1 M cacodylate

So for the solutions, we typically use:

- 2.5 ml cacodylate buffer (stock was 0.2 M)
- 1.5 ml tannic acid (stock was 4%)
- 0.25 ml glutaraldehyde (stock was 25%)
- 0.75 ml water

This brings the final total to 5ml

1. Fix 30 min.
2. Remove fix and wash with cacodylate buffer.
3. Wash 2x with ddH₂O
4. Fix with 2% osmium tetroxide on ice for 1 hour.
5. The pellet should now be black.
6. Remove osmium and wash 3x with ddH₂O

10. Incubate with 1% uranyl acetate for 1 hour
11. Remove UA and wash 3x with ddH₂O

12. At this point remove the pellet from the tube by cutting the bottom of the tube with a razor blade and teasing the pellet away from the tube with the dental tools. Put part or all of the pellet a glass petri dish.
13. Dehydrate in ethanol at steps of 50%, 95%, 2x 100% (15 min each).
14. Incubate in propylene oxide (PO) for 30 min.
15. Remove PO and incubate in PO/Epon (or other embedding media) at 1:1 ratio for 30 min (alternatively you can leave the sample in this overnight and let the PO evaporate.
16. Put in fresh Epon for 30 min, remove and put into Epon set up in embedding molds. Any orientation of the sample can be done at this time. When set and ready, put into the oven at 60C to polymerize.
17. When hardened remove and start sectioning.

Note: You can probably embed as you would for cells starting at step 13 and may not need the PO. This is just from my old notes.
Dehydration – ethanol series.

1.) Dehydrate with ethanol series of 20%, 50%, 70%, and 90% on ice 15 minute each.

2.) Dehydrate with 2X100% ethanol (dry) 15 minute each at room temperature.

Infiltration and embedding.

**Durcupan epoxy resin** - weigh & mix the following:

11.0 grams of A component
10.0 grams of B component
0.3 grams of C component
0.1 grams of D component

Mix 100 times with two wood applicators making sure no air bubbles are mixed in the resin.

Let resin stand for 20 minutes before each exchange.

3.) 50% ethanol (dry) : 50% Durcupan epoxy resin for 1 hr.

4.) 100% Durcupan epoxy resin for 1 hr. with lid off.

5.) 100% Durcupan epoxy resin for 1 hr.

6.) 100% Durcupan epoxy resin for 1 hr.

7.) 100% Durcupan epoxy resin for 1 hr.

Polymeration of Durcupan epoxy resin.

8.) Place in vaccum oven at -10Hg and 60°C to polymerize for 48 hours.

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Dehydration and embedding.pdf