**TEM immunolabelling**

Typical grid immunolabelling for a small number of grids (ie 10 or less)

**Things you need**

* several medium petri dishes
* 1 self-closing tweezer
* parafilm
* filter paper (cut into small triangles and 1 whole sheet)
* glass pipettes
* 2 washing 50 ml beakers
* timer
* grid staining cover
* NaOH pellets
* 2 % Uranyl acetate
* Lead citrate
* aldehyde block (ammonium chloride in TBST or PBST)
* protein block (3% BSA in TBST or PBST)
* wash (TBSHT or PBSHT)
* primary antibody
* secondary antibody

**Sample placement**

1. Put a square of parafilm inside each top lid of petri dish. Use the back of tweezer to flatten the edges of the parafilm. Droplets should be placed on the parafilm far enough apart they don’t coalese.

**Aldehyde block**

1. 10 mM NH4Cl in TBS for 10 minutes
   * 0.01 g NH4Cl in 20 mls distilled water or buffer
2. Put grids facing down into the droplets (i.e. shiny side up and specimen on the dull side facing droplet.

**Protein block**

1. 3% BSA in TBS for 30 minutes. Beware some antibodies stick to BSA. Check vendor instructions.

**Primary antibody**

1. Primary Antibody in TBS plus 1% BSA

**Wash**

Washing off the excess antibody is very important.

1. TBST 3 x 1 min

**Secondary antibody**

1. 1:50 in TBS plus 1% BSA for 1 hour.

**Counterstaining**

1. Put 8-9 NaOH pellets into one of the petri dishes for lead stain. Put lead citrate into a microcentrifuge tube and centrifuge it for some time before using.
2. Put droplets of uranyl acetate into another petri dish with a metal cover. \*\*Should only use uranyl acetate from the middle of the bottle so that precipitates will not be introduced to the grids.
3. \*\*Record the exact time each grid is placed in the UA droplets. For plant tissue, staining time is typically 30 min; for animal tissue, staining time is 12 min.
4. \*\*Put the grid staining metal cover on because uranyl acetate is light sensitive.
5. Fill up 2 washing beaker with d.d.w.
6. Once the staining time is up, pick up grid gently and dip grid into washing beaker. 1st washing beaker for 26 times, 2nd washing for 20 times. Or jet wash grid and put grid into a droplet of water while you deal with the other grids.
7. Use small triangles of hardened filter paper to dry the grids. Remember to use filter paper to dry the grid between the tines of the tweezer and push the grid onto a large piece of filter paper gently.
8. In the petri dish with NaOH pellets, put droplets of lead citrate.
9. Put grids facing down into the lead citrate droplets.
10. Stain grids in lead citrate for half the time it takes to stain in uranyl acetate (i.e. 15 mins for plant tissue and 6 mins for animal tissue). Timing is specimen specific – try it and see what works.
11. Refill the 2 washing beakers with d.d.w. after rinsing them 3 times with d.d.w.
12. Repeat washing steps after the staining time for lead citrate is over.
13. Put grids back into grid box.

Protein A-gold immunolabeling of tissue thin sections for electron microscopy

**Aldehyde block**

1. Incubation of the grids (sections down) on a drop of saturated solution of sodium metaperiodate. 60 min. at room temperature.

**Rinse**

1. Float the grids successively on four baths of distilled water (1 min. each).

**Protein block**

1. Transfer of the grids (always sections down) on a drop of PBS + 1% Ovalbumin, incubation for 5 min. at room temperature.

**Primary antibody labeling**

1. Transfer directly the grids on a drop of the antibody (anti - insulin antibody diluted with PBS at 1:200), incubation for 2 hours at room temperature.

**Rinse**

1. Float the grids successfully on five baths of PBS

**Protein block**

1. Transfer of the grids to a drop of PBS + 1% ovalbumin, incubation for 5 min.

**Protein A secondary labeling**

1. Transfer of the grids to a drop of the protein a-gold complex (dilution O.D. = 0.5 at 525 nm), incubation for 30 min. at room temperature.

**Wash**

1. Either by agitation or by jet wash with PBS.

**Rinse**

1. In distilled water. You must remove all the phosphate as it precipitates in UA. For this reason Tris is usually preferred.

**Dry**

**Stain with uranyl acetate and lead citrate**

**Comments**

* All along the incubations, the grids should float on the different solutions and we should avoid immersing them. Drying of the tissue sections should also be avoided until the final rinse in distilled water.
* Specifically use grids that have been dipped in a dilute formvar solution (about 0.1% in chloroform) and then dried on filter paper. The grids can either be dipped individually into the formvar and then placed on filter paper, or a whole canister can be dumped into formvar and each grid retrieved individually onto filter paper.
* This procedure produce a sticky coat on the grid bars and sections (epoxy, LR-white, etc.) really stay attached throughout all of the incubations.

Paraformaldehyde fixation for immunolabelling

**Buffer**

0.2 mono-dibasic Sodium Phosphate Stock Solution

* Solution A: 0.2M solution of NaH2PO4. H2O (27.6 g/L)
* Solution B: 0.2 M solution of Na2HPO4. H2O (28.4 g/L)

To make a 0.1M phosphate buffer at pH 7.3:

* 23 ml of soln A
* 77 ml of soln B dilued to 200 ml
* dilute to 200 ml