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MICROSCOPY 101

*We continue to appreciate contributions to this new publication feature. In addition to items relating to electron microscopy, we hope to publish more material on other microscopy techniques. To do this, and hopefully make the feature of increasing interest and value, we need **your** assistance.*

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EPON 812
Spurr's Resin Mixture Formula:

Prepare resin mixture the same day that it is to be used. Since infiltration takes more than one day, the stored resin mixture will begin to thicken by the second day (due to the presence of catalysts). Thus, fresh resin mixture should be prepared, and it is wasteful to prepare more than needed at any one time. Adjust volume according to the values given below. One gram equals approximately 1.2 mL.

Prepare resin mixture by weight using a top-loading balance that weighs accurately to at least two decimal places. Mix resins in plastic disposable beakers and add resins with strong pipet head to the beakers using Pasteur pipets. When mixing resins, do not worry about air bubbles. This will not be a factor in the infiltration steps. However, the final pure resin mixture for embedding should be free of air bubbles.

A.	Epon 812 (or substitute such as Epox 812)	5.0 gm.
	DDSA	2.8 gm.
	NMA	2.0 gm.
B.	ERL (From Spurr Kit)	2.2 gm.
	DER " " "	1.4 gm.
	NSA " " "	6.2 gm.
C.	DMP-30	0.2 gm.
	DMAE	0.2 gm.

Always use separate Pasteur pipets for each resin ingredient, and then dispose of them. Thoroughly mix ingredients in (A) above, together in a plastic beaker. Mix ingredients in (B) above, separately in another plastic beaker. Add ingredients from beaker (B) to those in beaker (A) and mix thoroughly. (Mixing can be done by using 2-3 wooden applicator sticks to completely stir the medium until no swirl marks can be observed.) Add the ingredients listed under (C) directly to the combined mixture in beaker (A). Stir in completely the DMP-30 first; then thoroughly mix in the DMAE. Tightly cover the beaker with Parafilm™ when not used. Store away from sunlight in desiccator. Storage time is limited to 24-30 hours at room temperature since it will become too thick to be effectively used. Always use resins which are not contaminated with moisture or organic residues.

When bottles are stoppered after pipetting needed amounts, clean the neck of each bottle with small amount of acetone on a cloth or Kimwipes - gently inject a moderate amount of Freon, CO_2 or other dry inert gas (from a duster can) into the bottle, and replace the cap without twisting too hard. Cover cap with tight strips of Parafilm™. Store in a cool (but not cold), dark location until next use. Most resin components last for several months to several years if properly stored at moderate temperatures and in an uncontaminated state.

After sectioning and staining, please remember that the best results are obtained when specimens are observed and micrographed in the transmission electron microscope as soon as possible.

Richard F.E. Crang, University of Illinois

Gold Toning for Silver Enhanced Immunogold Reacted Tissue:

Based on the method of Arai, R., M. Geffard and A. Calas, 1992. Intensification of labelings of the immunogold silver staining method by gold toning. Brain Research Bulletin 28:342-345.

Labeling tissue by the pre-embedding immunogold method and then silver enhancing the gold particles, gives beautiful staining ideal for both light microscopy and electron microscopy. However, after embedding the reacted tissue normally for TEM and cutting sections, it is common to find that most of the silver label has mysteriously disappeared. The reason Is that the osmium tetroxide used during the embedding procedure oxidizes the silver which then becomes soluble. Fortunately, only the subsequent uranyl acetate step seems to actually wash the silver salt out of the tissue - it does not matter whether or not the uranyl acetate is dissolved in water or alcohol. So, if embedding tissue without uranyl acetate en bloc staining gives usable sections, the problem of the disappearing silver will be solved.

An alternative approach is to gold tone the tissue before embedding. Gold toning will add considerable contrast to the tissue, so that subsequent section staining can be reduced.

Gold toning also makes the tissue rather "grainy", which shows up at higher magnifications, so keep reaction times to a minimum. The times quoted below have been found suitable for retinal tissue, which is around 300 μm thick, and for "average" sized silver grains. If the gold toned silver particles appear to have a hollow center, then lengthen the reaction times. Note: this recipe assumes that your tissue has already been immunogold-reacted and silver enhanced!

1. Silver enhance immunogold reacted tissue and wash as usual for your tissue.
2. Fix in glutaraldehyde. Tissue can be stored indefinitely in cacodylate buffer after this step.
3. Rinse in water for about 30 minutes.
4. Incubate in 0.05% gold chloride 30 minutes at 4°C.
5. Rinse in water 10 minutes.
6. Incubate in 0.05% oxalic acid 5 minutes at room temperature.
7. Rinse in water 10 minutes.
8. Incubate in freshly prepared 1% sodium thiosulphate for 1 hour.
9. Rinse in water for 10 minutes and embed normally for TEM.

Diana van Driel, Sydney University (Australia)

A Method for Making White Borders Around Black Text in Adobe PhotoShop:

Dry transfer letters had one big advantage over computers for labeling micrographs: they were available as white-outlined black letters, and so could be seen against any background. We have learned how to do this in PhotoShop. The following method assumes you are using version 4 on a Macintosh, although it Is possible in version 3 with minor modifications, and on a Wintel computer with slightly different keystrokes

1) With black as the foreground color, write the text (draw the arrow, etc.).