MICROSCOPY 101

We appreciate the response to this publication feature - and welcome all contributions. Contributions may be sent to Phil Oshel, our Technical Editor at:

Mr. Phil Oshel` (608)833-2885
PO Box 620068 eMail: oshel@terracom.net
Middleton WI 53562
(608)833-2885 ***PLease note NEW address***

Increased Preservation of Ultrastructure With LR Gold for Immunocytochemistry

The acrylic embedding media such as LR White, LR Gold, and others, have long been favored over epoxide embedding media for immunocytochemistry in cases where the antigen is sparse or vulnerable to the chemical activity of epoxy monomers. Since acrylics bond through tissue and not with it as epoxides do (1), Au labeling may increase sharply when acrylics are substituted for epoxides. Unfortunately one serious disadvantage of acrylics is their reduced ability to preserve ultrastructure.

LR White has emerged as the preferred embedding medium for immunocytochemistry, however it has been demonstrated that LR Gold produces better ultrastructural preservation than LR White (1,2, and unpublished observatiions). This improvement in the quality of fixation may become especially significant if osmium tetroxide is excluded during the fixation process. It is also important to note that LR Gold has increased beam stability when compared to LR White. This resistance to beam damage may be due to the aromatic bisphenol A, a polymer utilized in the production of epoxides. Bisphenol A is internalized in the methyl and butyl methacrylate formulation of LR Gold, but not in LR White embedding media[1]. This suggests that investigators who use LR White for immunocytochemistry should consider the advantages of LR Gold.

1 Newman, G.R., and Hobot, J. A., Resin Microscopy and On-Section Immunocytochemistry. Springer Verlag, Heidelberg (1993) pp 132, 37, 36.

2 Berryman, M.A., Rodewald, R.D., An Enhanced Method for Post-embedding Immunocytochemical Staining Which Preserves Membranes, J. Histochem. Cytochem. Vol. 38, No. 2, pp159-170, 1990

Hildegard H. Crowley, University of Denver

Osmium as an Immunostain Enhancement

Since we do our paraffin-section DAB immunostaining in an EM lab, we often use osmium to darken the reaction product. We simply put a drop of our standard 1% cacodylate-buffered osmium tetroxide on each section in a hood and rinse it off in running tap water after about 2 seconds. It makes light DAB reaction product pretty dark brown, and adds a light brownish tint to the rest of the section. The reaction increases the contrast quite a bit.

This is expensive, because of the cost of Osmium, and requires proper means of disposal of the waste Osmium, but an EM lab will already have both the Osmium and a means to dispose of it. As well as a fume hood! Without good ventilation, you will start to notice rings around lights very quickly!

We mentioned this method in our paper:

Hawkins, H.K., M.L. Entman, J.Y. Zhu, K.A. Youker, K. Berens, M. Dore, and C.W. Smith. 1996. Acute inflammatory reaction after myocardial ischemic injury and reperfusion. Development and use of a neutrophil-specific antibody. Am. J. Pathol. 148(6):1957-1969.

Hal Hawkins, University of Texas Medical Branch

A Hint for Fixing Tissues for Better Staining

For beautiful colors in near perfectly preserved cells and connective tissue, immerse carefully cut pieces, no more than 2 mm in least dimension, *overnight* in either Heidenhain's SUSA or Helly's fluid or Zenker's fluid without the acetic acid (which is the same as Helly's without the formalin). Wash thoroughly in water (tap water is OK) for several hours (overnight is sensible) and then dehydrate, clear, infiltrate, embed and cut paraffin sections

After dewaxing and hydrating the slides, put them in an iodine solution (Lugol's, Gram's or 0.5% I_2 in 95% ethanol), then in sodium thiosulfate (2-6%) to remove the brown iodine color. Wash in running tap water for a minute to get rid of the chemicals. (This step Is a must after any mercury-containing fix.)

Stain by any good, interesting method (meaning almost anything other than hematoxylin & eosln).

Compare the result with that obtained with routine formaldehyde-fixed specimens. When comparing, think especially about what you believe to be the most important features seen with the microscope.

John A. Kiernan, The University of Western Ontario

A Procedure for LW Polymerization of Lowicryl or Similar Methacrylates

1. Use polyethylene fiat embedding molds. These molds are made from the same type of material as BEEM capsules. The polyethylene molds are
transparent to the UV light, making the polymerization much more even.
2. Place the mold on a piece of cardboard that is wrapped in aluminum foil. The cardboard should be larger than the mold. This makes moving and transferring the mold much easier and less messy (see below).
3. Overfill all of the cavities in the mold to give a "positive meniscus" at each position. If some of the cavities don't have specimens in them, go ahead and fill the cavities anyway.
4. Cut a piece of Parafilm that is slightly larger than the mold. Then, beginning at one end of the mold, gently lay the Parafilm down on top of the mold. As you do this, the excess resin will run out of the mold cavities and get trapped under the Parafilm. Gradually lower the Parafilm down toward the other end of the mold, allowing the resin to fill the space between the mold and the Parafilm. This seals the complete top of the mold. The resin won't dissolve the Parafilm, but it will make it soft. Thermanox (plastic) cover slips can be used instead of Parafilm. Just lay the coverslips on top of the cavities, letting the excess resin mn run along the edges.
5. You can use tissues to absorb the excess resin as it runs down the outside of the mold. (**CAUTION:** Be sure to wear gloves at this step, and at all steps when working with methacrylate resins - or any resin, for that matter!).
6. Transfer the filled molds to your polymerization apparatus. I make polymerization chambers out of cardboard boxes. Just make two cutouts: one on the top for the UV lamp, and one on the side for a door. Note! To make the polymerization even, cover all of the inner surfaces of the box with aluminum foil.
7. Let everything equilibrate at low temperature for 15 – 20 minutes, turn on the UV lamp, and you're on your way to polymerized resin!
8. If you see "bubbles" or vortex-like "swirls" in the polymerized blocks,

Continued on following page