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:

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PLease note NEW address

Mold Release Solution

We originally (long ago) kept our molds (at that time they were five-sided metal "boats" of various sizes which had been manufactured by the machine shop) in soapy water, and merely drained out the excess fluid just prior to filling them with paraffin. After the paraffin was sufficiently hardened, we plunked the blocks in ice water, and the paraffin would eventually float out of the mold. It worked very well, but was messy, and occasionally block surfaces were minimally deformed due to soap bubbles remaining in the corners of the molds. When my level of distress over the illogically ever-increasing price of scientific supplies reached the unbearable, I came up with the following soap spray, and successfully used it with Tissue-Tek molds for years.

Mix a solution of 5% green dish washing soap (such as Palmolive) in 50% Ethanol, then put it into a pump spray bottle (available form any housewares department). This works *at least* as well as the outrageously expensive stuff sold as 'Mold-Release Spray", and contained no CFC's or other "evils".

Joanne Lahey, Battelle Duxbury Operations

Negative Stain Procedure For Fixed Viruses

1) Put a drop of fixed virus suspension onto your coated grid for about 60 seconds. Then very slowly suck up virus suspension with a Whatman filter paper (cut filter paper into triangles, with sharp arrow point like tip).

2) Then float grid containing virus onto a drop of 0.05 M sodium cacodylate buffer (2 x 2 minutes wash).

3) Wash with double distilled water (2 x 2 minutes)

4) Negative stain for 30 seconds with 2% Phosphotungetic Acid.

5) Dry and view

Vijay H. Bandu, University of Natal, South Africa

A Suggestion for Increasing the Intensity of Staining in Hydrophobic Resins Such as Spurr's

The stain solution should be made up at high pH so that protons in solution don't compete with stain molecules for binding sites:

0.5% toluidine blue in 0.1% sodium carbonate, pH 11.1

The metachromicity of toluidine blue depends on water molecules being present, so mounting media like Entellen (Xylene base?) ruin this effect. My trick is to exhale fairly forcefully onto the sections just before adding a solvent-based mounting medium. This adds just enough water so that colors appear as desired. If you do enough slides at once, the hyperventiilation

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combined with the effect of breathing mountant fumes gives one a special feeling all over. I have not noticed fading in slides prepared this way.

You should be able to make up other stain solutions to high pH in a similar fashion.

C. John Runions, Cornell University

Stacking Collodion Layers When Making Films

4% Collodion (Parlodion, nitrocellulose in amyl acetate) is good for making films, but Collodion solutions also have a wonderful additional property in that you can stack the layers.

Gently place one drop on the water surface, as the drop spreads it reaches maximum spread and then 'bounces" back a little. Right when it bounces back add one more drop to the middle.

The second drop spreads on top of the first, giving a thicker film. Use the color index (just like sectioning) to determine approximately how thick the film is.

Keep adding drops of the Collodion until you get a color/thickness you like. I usually use 2 drops.

When casting collodion on water, it is important that you use a large enough container so that the solution can spread to its full extent, and not be limited by running into the sides of the container. When it hits the side(s) of the container, the film will pile-up in that area, resulting in an uneven film thickness.

Any circular dish seems to work, but I prefer using 10" x 12" Pyrex baking dishes purchased from local home stores (*i.e.,* K-mart, Walmart, etc.). They are very cost effective ($3 to $9 US), and have nice thick walls which stand up nicely to general lab abuse.

Richard E. Edelmann, Miami University

Processing of Skin Tissue for Light and Electron Microscopy Embedding in Epon 912

(italics are for a rapid procedure):

Skin is a problem tissue which we deal with routinely. Here is our procedure:

1) Place newly received tissue in 1/2 Karnovskys fixative overnight (*2 hours to overnight*).

2) Take Epon 812 mixture out of refrigerator (let it sit under the hood for at least 2 hours before opening the container). This mixture is a combination of DDSA, NMA. and EMBED 812 (48% of 812, 31% of DDSA, 21% of NMA).

3) Rinse biopsy in 0.1 M sodium cacodylate buffer x 2, 15 minutes each rinse (*5 to 10 minutes*).

4) Post-fix in 1% osmium tetroxide, 1.5 hours (mix 1:1, 2% OsO_4 with 0.2 M sodium cacodylate buffer) (*20 minutes*).

5) Rinse in distilled H_20 x 2, 15 minutes each rinse (*3 to 5 minutes*).

6) En-bloc stain with 1% uranyl acetate for 1.5 hours (*20 minures*).

7) Dehydrate through an ascending EtOH series:
35% x 2 (15 minutes each) (*5 minutes x 2*)
70% x 2 (15 minutes each) (*5 minutes x 2*)
95% x 2 (15 minutes each) (*5 minutes x 2*)
100% x 2 (30 minutes each) (*10 minutes x 2*).

8) Note! Each resin/catalyst/propylene oxide mixture is made fresh, and the pure resin/catalyst is made fresh. Add the catalyst to the Epon 812 mixture (0.2 mL of DMP3O per 10 mL resin). Stir slowly for 10 minutes.

9) Clear biopsy in Propylene oxide x2, 15 minutes each rinse (*10 minutes x 2*).

10) Infiltrate by placing biopsy into:
3:1 mixture of Propylene oxide: Epon for 3 to 4 hours (*30 minutes*)
2:1 mixture for 12-16 hours (overnight) with caps off (*30 minutes*)
1:1 mixture for 12-16 hours (overnight) with caps off (*60 minutes*).

11) Place biopsy into an embedding mold with fresh 100% Epon for 9 hours, then place mold into 60°C oven for curing (24 to 48 hours).

(Epon 812 can be substituted with either EMBED 812 (EMS). PolyBed 812 (Polysciences), Medcast or Eponate 12 (Ted Pella). All have the ingredients DDSA, NMA, and OMP 30.)

Bob Underwood. University of Washington

Hints on Surface Decalcification of Paraffin Embedded Tissues:

To surface decalcify paraffin embedded tissues containing calcium deposits, the trimmed block is immersed face down in a few milliliters of acid decalcifying fluid contained in a shallow dish for a minute or so. Rinse the acid away with water, and/or neutralize the acid with saturated lithium carbonate or 5% sodium bicarbonate, rinse, cool block and resume sectioning.

Neutralizing the acid will help prevent acid corrosion of delicate metal parts on microtomes and microtome blades.

Any commercially or in-house prepared decaicifying solution containing hydrochloric. nitric or formic acids may be used. Strong acid decalcifiers (HCl and nitric) will decalcify rapidly, and can be diluted with water 1:1 if needed. Use safety precautions to prevent exposure to acid fumes or skin and eye contact.

Surface decalcification is not an uncommon practice on breast or other soft tissues with tiny calcium deposits. However, if residual calcium in an underdecaicified bone sample is the problem, then decalcification end point testing is advisable to insure the bone *is* completely decalcitied.

Gayle Cailis, Montana State University

Electron Microscope Technician

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