Goldner's Stain for Methylmethacrylate Embedded Bone Sections

Goldner's Trichrome Stain, preparation:

Weigert's Iron Hematoxylin:

Solution A:

Hematoxylin (Sigma H3136): 10 g

100% Ethanol: 1 L

Solution B:

30% Ferric Chloride (Sigma F2877): 40 mL

Hydrochloric Acid: 10 mL

Distilled Water: 950 mL

Mix equal volumes of solutions A & B

Ponseau-Acid Fuchsin:

Ponseau S (Sigma P3504): 10 g

Acid Fuchsin (Sigma F8129): 5 g

1% Acetic Acid: 1 L

Mix well and filter.

Phosphomolybdic Acid-Orange G:

Phosphomolybdic Acid (Sigma P7390): 20 g

Distilled Water: 1 L

Orange G (Sigma O1625): 1 L

Dissolve phophomolybdic acid then add Orange G. Mix well and filter.

Light Green:

Light Green (Sigma L53B2): 2g

Acetic Acid: 2 mL

Distilled Water: 1 L

Staining Procedure:

Remove methylmethacrylate in ethylene glycol monoethyl ether acetate (Fischer E181-4): 15 minutes X 2

hydrate sections to distilled wafer

Weigert's Iron Hematoxylin: 15 minutes

distilled water rinse

tap water: 15 minutes

distilled water rinse

Ponseau-Acid Fuchsin: 15 minutes

I % Acetic Acid rinse X 2

Phosphomolybdic Acid-Orange G: 8 minutes

I % Acetic Acid rinse X 2

Light Green: 15 minutes

1% Acetic Acid rinse

Dehydrate, clear & coverslip.

Results:

Nuclei blue. cytoplasm red, mineralized bone and collagen green, osteoid red, erythrocytes orange.

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Using Borohydride to Quench Autofluorescence of Glutaraldehyde

If glutaraldehyde was used in fixing tissues, then it may be advantageous to quench extraneous aldehyde groups with 1% sodium borohydride ($NaBH_4$) (Eldred *et aL*, 1983, J. Histochem. Cytochem. 31:285), which is a particularly strong reducing agent *(use with caution).* Borohydride can eliminate most tissue autofluorescence, thus reducing background in light microscopy immunocytochemical studies involving fluorescent markers. It has been suggested that borohydride may partially restore antigencity afler glutaraldehyde fixatlon by reducing Schiff bases (carbon-nitrogen double bonds) that can be formed when glutaraldehyde reacts with free amino groups on proteins; the reduced bonds are less rigid, and the increased mobility may restore some of the antigenicity.

Borohydrlde treatment can be carried out on pieces of tissue after fixation and the overnight buffer wash (Eldred *et al.*, 1983). Use 1% sodium borohydride in phosphate buffered saline (PBS) for 30 minutes at room temperature. The solution should be freshly prepared from sodium borohydride powder that has been stored protected from moisture. The tissues will bubble vigorously as hydrogen gas leaves them, which will be worrying, but doesn't seem to damage the tissues. The treatment is followed by a wash of 2 x 30 minutes in PBS.

To use borohydride as a quenching agent during an light microscopy immunocytochemical run, put a drop of 1 % sodium borohydride on each tissue section and leave it for about 10 minutes. Then wash in a Coplin jar of PBS.

For convenience, 10 mg aliquots of sodium borohydride powder can be stored in microtubes at -20° C in a plastic box containing silica gel desiccant. When you need 1% borohydride, add 1 mL of PBS to a tube and vortex briefly (CAUTION: open the microtube promptly after vortexing, or the hydrogen gas generated as the powder goes into solution will pop it open, possibly causing a spill).

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An Alternative to Agar Embedding for Cell Suspensions

We have had good luck with a trick we picked up from the cryofixation folks. We make wire loops out of very fine copper wire (36 gauge) and coat the loop with Formvar. The diameter of the loop can be a few millimeters or up to 7mm (we have never tried larger).

Cells are then collected on the Formvar. For example, if the cells are in a small volume of solution, go "fishing" with the loop. The Formvar surface can be made more sticky by pretreatment with polylysine.

Then place a second Formar coat over the loop, thus trapping the sample between two Formvar films. Be sure that there isn't too great a puddle of liquid on the loop for this step. Some fiddling will be needed.

We cast small rectangles of Formvar on water, with the narrow side of the rectangle a bit wider than the loop diameter, and the long side of the rectangle a bit longer than twice the loop diameter.

Then very quickly dunk the loop with the samples onto the Formvar rectangle. Line up the loop with the middle of the rectangle, so it makes two squares. and plunge at right angles to the water.

The Formvar rectangle just snaps to the loop, with cells trapped inside. The Formvar is readily permeable to fixative, to dehydration agents. and even to paraffin.

At the end of the "day", excise the loop with a razor. In fact, if you really get 36 gauge copper, it can just be cut through with a double-edged razor blade.

More detailed information may be found in the following two citations:

Baskin, T.I., D.D. Miller, J.W. Vos. J.E. Wilson, and P.K. Hepler. 1996. J. Microsc. 182:149-161. This article has information about wire loops (such as polylysine coating protocol), and a few other things.

Baskin, T.I. and J.E. Wilson. 1997. Plant Physiol. 113:493-502. This article describes the double formvar sandwich trick.

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