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Butyl-methyl-methacrylate for Immunocytochemistry Through the Light Microscope

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The use of methacrylate monomers for embedding has a venerable history in microscopy. Many formulations have been developed over the years for various purposes, ranging from standard TEM observations to low-temperature embedding. Key parameters include the length of the hydrocarbon chain and the presence and kind of cross linking reagent. In the mixture of butyl and methyl methacrylate (BMM) described here, the monomers are relatively short-chained and there is no cross linker at all. This gives the polymerized material a softness that makes it rather unsuitable for TEM, but on the contrary allows the embedment to be removed after sectioning by a brief incubation in acetone. The latter property is good for immunocytochemistry because loss of the embedment means greater access for the antibody to the antigen. BMM generally preserves structure better than paraffin or glycol-methacrylate, and for this reason is a useful choice for light-level immunocytochemistry, particularly when sub-cellular resolution is desired.

Methacrylate polymerizes via a free-radical based mechanism. Early attempts to use BMM for immunocytochemistry were hindered because the free radicals attacked the sample, compromising its antigenicity. I found that adding a free-radical scavenger, dithiothreitol (DTT), had the happy result of preserving antigenicity without stopping polymerization (Baskin *et al.* 1992). Polymerization can be initiated by either heat or UV. Because the reaction is quite exothermic, heat-induced polymerization has the potential to be damaging. Therefore, the protocol below uses UV and has polymerization go at 4˚C.

My work uses BMM for plant tissue; colleagues have had good luck using it for animal tissue. I have not studied whether there are advantages of BMM that are plant specific.

Resin mixture

For 50 mL, use:

- 40 mL butyl-methacrylate
- 10 mL methyl-methacrylate
- 250 mg benzoin ethyl ether
- 77 mg (10 mM) DTT

Mix the methacrylates and add the DTT. Let stand 1 to 2 hours. Mix gently.

Bubble nitrogen gas through mixture for *ca.* 20 min to remove dissolved oxygen.

Add catalyst and mix gently.

The mixture can be stored at minus 20˚C for many months. The methacrylates are ‘ordinary’ grade, and contain a stabilizer, which is not necessary to remove.

Infiltration

The resin mixture generally infiltrates well, but some samples can be problematic. For our samples (small plant roots), we use a graded ethanol series (25%, 50%, 75%, 90%, 95%, 3 x 100%, 30 minutes each), three graded ethanol/BMM steps (3:1, 1:1, 1:3 ethanol to BMM; 2 to 4 hours each), and three changes in 100% BMM (2 to 4 hours each, one of them overnight). BMM is also miscible in acetone and methanol.

Embedding

BMM is extremely volatile and oxygen inhibits the reaction: therefore, embedding should be done in sealed containers or in a hood. Note that the reaction is probably NOT as oxygen sensitive as that of the London resins. The optimal UV for polymerization is 365 nm. High intensity is not required. We use a 15 W bulb with our samples about 10 cm distant. We stand flat bottom “BEEM” type capsules on a piece of clear acrylic, held over the bulb. In this way, polymerization goes from the bottom up, allowing any bubbles that form to escape upward. It can be useful to minimize direct light from reaching the samples or the polymerization can be uneven. The box with the samples is place in the cold room (4˚C). Polymerization is usually complete after 4 hours. Samples are then put in the hood with the caps off so that any unincorporated monomer can escape.

Sectioning

The resin can be trimmed and sectioned using standard techniques. We find that the blocks are brittle and that in the early stages of trimming, care must be taken to avoid fracturing the block. Lowering the DTT concentration will make the blocks less brittle. Various combinations have been tested by Palmer *et al.* (2001). We cut 1.5 to 2 μm thick sections, on a glass knife, dry. It is also possible to cut sections wet, and then sections can be cut much thinner. Sections are transferred to small drops of water on slides, exposed to 60˚C for a minute to help spread the sections. Heat pens spread sections more aggressively.

We usually coat slides with 3 – aminopropyltriethoxysilane.
Make a 2% solution of the above in acetone; dip slides in for 1 min
dip slides in 100% acetone for 1 min
dip slides in H_2O for 1 min
dip slides again in H_2O for 1 min and let air dry.
Slides are sticky indefinitely. However, I think the sections would stick pretty well to ordinary slides.

Staining

Sections are extracted by placing the slide in fresh acetone for 10 minutes. **Note:** acetone has a limited ability to extract methacrylate: one staining jar (*ca.* 75 mL) of acetone can do two slides with few dozen sections each, and after that the acetone becomes demonstrably less effective.

Remove the slide from the acetone and quickly place it in phosphate-buffered saline (graded re-hydration seems not to be necessary). Note that acetone removes most of the embedment but an insoluble residue remains. The slide can then be handled as per your favorite immunocytochemistry procedure.

Citations

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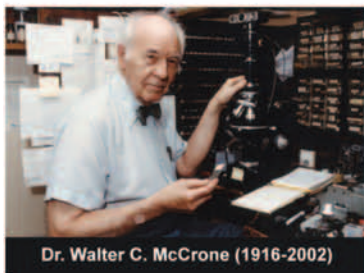

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