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Guide to Plastic Sectioning and Embedding

Why use methylmethacrylate (MMA) to embed?

Methylmethacrylate embedding has been used for many decades in light microscopy specimen preparation and was the first resin in wide use for electron microscopy sample preparation. Its favorable properties include low viscosity for complete and rapid infiltration (relative to other resins) and the high hardness of the resulting polymer, sometimes referred to in the literature as polymethyl- methacrylate (PMMA). These properties allow thinner sections to be cut providing higher quality morphological data and harder samples to be processed. In fact, undecalcified bone is almost entirely done this way.

Hardness of the sample is only one criteria for selecting MMA, since the resulting block hardness can be controlled to make it suitable for softer tissue. If you want to remove the plastic from the sections you've cut, MMA can be readily dissolved by several solvents. Initiator systems are now available which allow faster processing (a day or two) at lower temperatures making immunohistochemical determinations possible.

If this material appears to be a desirable embedding media for you, read on. You'll find protocols for its use, from embedding to staining, and detailed information about the unique products Delaware Diamond Knives supplies to make these tasks yield better results more easily.

Embedding Protocols

Excessive tissue, muscle, skin, tendon and fat must be removed. Best embedding results if you can split the bones below the secondary spongiosa. This allows for more rapid and complete fixation, dehydration and infiltration.

Fixation @ 4° C:

Select from the following recommended alternatives:

1. 40% ethanol for 2 to 3 hours minimum followed by
   70% ethanol for 7 days. Change the alcohol solution daily.
2. Schaffer's solution for 24 hours. This is good for small
   samples like iliac crest biopsies.
3. 4% paraformaldehyde for 7 hours maximum is good for
   enzyme and immunohistochemistry.
4. 2.5% gluteraldehyde in 0.05 mol/l PBS and 0.1% sucrose
   for at least four weeks.

Dehydration @ 25° C and vacuum:

Dehydration of the fixed tissue is carried out in increasing concentrations of ethanol. Each solution should be changed once and times of dehydration should increase with specimen size. Match the dehydration schedule number with the fixation protocol selected from above.

1. 80% then 96% ethanol for 24 hours minimum.
   Remember to change solutions each 12 hours.
2. 80% then 96% ethanol for 24 hours minimum.
   Remember to change solutions each 12 hours.
3. Rinse 3 or 4 times for 15 min. each with PBS (Sorensen's)
   followed by 50, 70, 80, and 96% ethanol for 24 hours minimum.
4. Rinse 3 or 4 times for 15 min. each with PBS (Sorensen's)
   followed by 50, 70, 80, and 96% ethanol for 24 hours minimum.

Follow these schedules with 100% ethanol for 48 hours minimum changing solutions each 12 hours. Please note that

these are maximum times for large samples. Smaller samples can use significantly reduced times, e.g. iliac crest biopsies can use steps as short as 2 hours with solution changes on the hour.

Defatting @ 25° C and vacuum:

Xylene is usually used in a clearing step at this time. Small samples can be done in two steps of two hours each. Large samples should be done in two steps of two days each.

Infiltration @ 4° C and vacuum:

The mixture for infiltration is made of 94% MMA, 5% Plasticizer and 1% Activator. All percentages are in weight percent. Cover the samples for two hours, replace the solution and leave overnight for small samples and two days for large samples.

If you plan to saw and/or grind sections, the proportions should be changed to make the blocks slightly harder. Instead use 97% MMA, 2% Plasticizer and 1% Activator. After polymerization, these blocks will react more favorably to the heat and forces generated with the saws and grinding apparatus available today.

Embedding @ 30° C and vacuum:

The same proportions of components are used for embedding as for infiltration. Use glass, PVC or Teflon embedding molds that can be sealed securely. After covering your sample with embedding solution, draw a vacuum on the mold for 30 minutes. Break vacuum and secure the mold's lid. Place the mold in a 30° C water bath overnight for complete polymerization.

Sectioning and Mounting Protocols

Sectioning is best done with a rotary or sledge microtome. The features to look for are retraction of the specimen on the return stroke and motorized motion of the sample relative to the knife edge. Retraction is needed to keep the hard sample from brushing against the knife edge on the return stroke, damaging the edge and block face. Motorized sample movement is desirable to get more reproducible cutting speed and force.

The best sections are obtained on these microtomes with a tungsten carbide knife. Depending on the block size and knife quality, section thickness in the range of 2 to 10 microns is possible. If you get good looking sections with a dry block face, continue without making any changes. If not, keep the block face wet by applying 30% ethanol. This softens the block face

just enough to help prevent chatter or formation of microcracks.

Most procedures call for mounting your sections on slides before staining. The exception may be when staining sawn sections since these retain enough strength to be held in tweezers and hand- dipped in the various staining solutions (followed directly by coverslipping to slides). Mounting thinner sections to slides before staining also helps to prevent folds from developing in the sections. Here are three protocols:

Haupt's Solution

Procedure:

1. Use a positively charged clean slide, the Fisher Superfrost +, for example.
2. Place a thin film of Haupt's Solution on the slide.
3. Add a few drops of 50% ethanol to soften the section.
4. Place the section on the alcohol, trying to flatten it out as much as possible with tweezers, applicator stick, or a sable brush.
5. Cover the section with a piece of polyethylene film (Saran wrap).
6. Add a piece of filter paper.
7. Roll excess fluid from the stack with a wallpaper seam roller.
8. Place in a stack with other slides in a slide press overnight. Room temperature to 56° C can be used.
9. In the morning, break apart the stack of slides. Remove the plastic from the sections, if necessary, and begin staining protocols.

Solutions:

Haupt's Solution

- 500 ml. of distilled water. Heat to 30° C.
- 5 gms. of gelatin
- 10 gms. of phenol crystals
- 75 ml. of glycerine
- Stir until fully dissolved and filter.

Source:

Mary (Taffy) Hooser HT(ASCP)
Department of Anatomy
Indiana Univ. School of Medicine
Indianapolis, IN

Silanized Slides

Procedure:

1. Dip slides in 2% silane / acetone solution for one minute.
2. Dip slides in 100% acetone for one minute.
3. Dip slides in double distilled water for one minute.
4. Repeat step 3 with agitation.
5. Air dry.
6. Apply sections on a drop of water on the slide.
7. Dry on 60° C hot plate for 2 - 5 minutes.
8. Air dry at room temperature overnight.

These silanized slides can be stored at room temperature for months without any problems.

Solutions:

The silane material comes in a liquid form from Sigma. The full name is 3, aminopropyltriethoxysilane.

Source:

Tobias Baskin
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109 Tucker Hall
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Citation:

Angerer, L. M. & Angerer, R. C. (1991) Localization of mRNAs by in-situ hybridization. Functional Organization of the Nucleus: a Laboratory Guide. Methods in Cell Biology, Vol 35, (ed. by B. A. Hamalko & S. C. R. Elgin), pp. 37-71. Academic

Press, San Diego.

Haupt's Coated Slides

Procedure:

1. Coat your slides with Haupt's Solution by dipping your acid-cleaned slides in a container of Haupt's solution for 2 - 5 minutes. Stack in a suitable dust-tight container. Dry in 37° C oven overnight.
2. Warm the slide on slide warmer to 35 - 40° C.
3. Make small pool of 70% alcohol.
4. Float section on alcohol pool with minimal manipulation to remove folds/bubbles.
5. Let stretch for a few minutes.
6. Add plastic foil such as Kisol Foil.
7. Drain off excess alcohol.
8. Roll with thumb.
9. Stack with other slides in a press overnight in 56° C oven.

Solutions:

See the recipe for Haupt's Solution given above.

Source:

Dawn Lundin
Henry Ford Hospital
Bone & Joint Center
Detroit, MI

Removing Plastic and Rehydrating Sections

Many staining protocols require that you remove the MMA and rehydrate your mounted sections in order to provide deeper penetration (and greater contrast) for your stains. If you ever have this need, follow one of these protocols:

1. Methoxyethyl acetate (Aldrich Chemical): Change solutions three times with 15 - 30 minutes between changes. Procedure

is done at room temperature and sections may be allowed to soak overnight.

2. Ethylene glycol Monoethyl ether (Fisher): Change solutions three times with 15 - 30 minutes between changes. Procedure is done at room temperature and sections may be allowed to soak overnight.

3. Acetone: Change solutions three times with 15 - 30 minutes between changes. The last change should be left overnight. Procedure is done at 60° C.

Rehydrate your sections by using the following series:

100% alcohol, two times, 15 minutes each.
95% alcohol, two times, 5 minutes each.
80% alcohol, three times, 5 minutes each.
70% alcohol, three times, 5 minutes each.
100% distilled water.

Your sections are now ready for staining or immunlabeling.

Source:

Judi Lloyd
Univ. of Pennsylvania
New Bolton Center
Kennett Square, PA

Staining Protocols

This is the current collection of stains for methylmethacrylate sections. We are actively searching for new stains to add to this collection and want to add your favorite.

Von Kossa Stain for Calcium

Hematoxylin and Eosin

Toluidene Blue

Tetrachrome Stain

(Microwave)

Goldner's
Modified
Trichrome

Movat's
Pentachrome

Giemsa Surface
Stain

Tartrate Resistant
Acid
Phosphatase

Alkaline
Phosphatase

Misc. FAQ's

1. My block is full of bubbles! How do I avoid this?

Blocks full of bubbles are due to the heat of polymerization causing the monomer to boil. Bubbles are captured when this boiling occurs right at the end of the polymerization, when the monomer solution is extremely thick and viscous. It is not uncommon for these bubbles to be located at or near the tissue since the tissue acts as a nucleating site for any bubble formation during boiling.

We recommend that you polymerize in a water bath in order to provide a large heat sink to absorb this heat that is generated. (A trough of water in a low temperature oven works, too.) You might also consider, especially if you are creating a large block (over ~100 ml.), polymerizing in stages or reducing the amount of initiator used in the mix. Lower volumes, reduced initiator concentration and lower temperature of polymerization will all help to reduce or eliminate your bubble problem.

2. The cortical and trabecular bone in my sections is cracked!

What did I do?

Cracking can be due to two causes:

If when examining the cut block face you can see cracks in the plastic and sample, you are probably using alcohol of too high a concentration during sectioning. Cut the concentration and then stretch your sections with higher concentration alcohol during the mounting step. While you can refill these cracks with resin and repolymerize, the cracks now present in the tissue will only be filled, not truly repaired.

If the cut block face looks alright, the cracks in the tissue are probably due to overmanipulation during mounting on slides. The general rule is to handle your sections as little as possible. Change mounting protocols to find one that minimizes your handling of the sections. Be sure that the plastic film and not the filter paper is in contact with the section when stacked and pressed. Keep in mind that the more you handle your sections, the more opportunity there is for damage.