GMA Kit for LM Applications
USE INSTRUCTIONS

SPI Supplies
206 Garfield Avenue,
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Low-Acid GMA Water Soluble Embedding Kit for Light Microscopy Applications

Instructions for Use of SPI #02640-AB

CAUTION:

Thoroughly read all instructions and warnings on container labels prior to use of this kit.

Introduction:

Characteristics which make low-acid GMA suitable as an embedding medium for light microscopy (LM) include:

- Low-Acid GMA polymer resists the uptake of basic stains, thereby reducing nonspecific staining and false histochemical reactions in sections of biological materials.
- The GMA monomer is miscible with both water and ethanol.
- GMA infiltrates and polymerizes without the complete removal of water.
- Both soft and hard tissues are well infiltrated and supported by the GMA medium.
- Thermal artifact can be reduced due to low temperature infiltration and embedding at 0-4°C.
- Sections of GMA embedded tissue are readily cut at 0.5 to 3.0 micrometers using steel or glass knives
- Enzyme digestions, a variety of stains and immunological localizations may be performed on thick sections of GMA-embedded tissue sections without removal of the plastic.

Formulations: (Cole and Sykes, 1974)

A. Unpolymerized GMA:
100.00 ml glycol methacrylate (Low-acid content).
**0.15 g benzoyl peroxide.
5.00 ml polyethylene glycol 400 or 200.

Mix thoroughly and store in a brown glass bottle in a freezer. The solution is stable for 6 months or longer. Allow solution to come to room temperature before opening to prevent condensation from contaminating the solution.

NOTE:
This mixture is used to make up both the alcoholic and aqueous dilutions and is used without dilution for final infiltration. It is NOT used as a substitute for pre-polymerized GMA or as the stock solution from which to make pre-polymerized GMA.

B. Pre-Polymerized GMA:
100.00 ml glycol methacrylate
**0.15 g benzoyl peroxide

Mix well and then pour enough of the solution into a 125 ml or 250 ml Erlenmeyer flask (Pyrex) to form a shallow (5 to 7 mm) layer. Heat slowly over a Bunsen burner with constant swirling. The solution is polymerizing when the color deepens (to yellow-orange) and begins to thicken. When the solution begins to polymerize, immediately plunge the flask into an ice water bath and swirl vigorously until the solution is cooled to the temperature of the ice bath. Polymerization is an exothermic reaction and once polymerization begins, rapid cooling is imperative to prevent the VERY RAPID complete polymerization in the flask. If the proper viscosity is not reached after the initial heating and cooling, repeat the procedure as many times as necessary to reach the desired end point. Properly pre-polymerized GMA should have the viscosity of thick syrup at ice water temperature. That is, small air bubbles within the solution should rise very slowly to the surface.

Once the proper viscosity is obtained, allow the solution to come to room temperature and then add 5.0 ml of polyethylene glycol 400 (or 200) for each 100 ml of pre-polymerized GMA. Mix well (1 hour on magnetic stirrer). The pre-polymerized solution may be stored in a freezer for 2 months or longer. The purpose of pre-polymerization is to "pre-shrink" the GMA. Unpolymerized GMA may shrink about 5% of its volume; pre-polymerized GMA should shrink less than 1% of its volume.

***SPECIAL PRECAUTION:** All mixing and/or heating operations must always be performed in a fume hood. The liquid GMA components may cause contact dermatitis _ allergic reactions in some individuals. The use of latex surgical gloves seems to help prevent the dermatitis. The polymerized GMA appears non-toxic. ******Avoid loss of water content from the benzoyl peroxide as the dry material is considerably more hazardous and will burn vigorously. Store below 100°F (38°C) to maintain activity.

Fixation, Dehydration, Infiltration and Embedding

Fix and rinse small (2 mm thick slices) pieces of tissue.
(A number of fixation procedures may be used, but those recommended include: 1 percent buffered glutaraldehyde, and 10 percent buffered formalin.

Two methods of dehydration/infiltration are commonly used:

A. The first requires no alcoholic dehydration and is recommended for all aqueous-based fixatives.

B. The second method includes alcoholic dehydration prior to infiltration and is recommended for-non-aqueous-based fixatives.

METHOD A: (aqueous)

1. Immerse well-rinsed tissue in 85 percent aqueous GMA--30 minutes. The solubility of GMA in water may be increased by reducing the temperature to 4-8°C.

2. Immerse in 97 percent aqueous GMA--30 minutes. Then go to step #3 below. Dehydration and infiltration may be carried out in an ice water bath or refrigerator to minimize loss of temperature-labile components of cells.

METHOD B: (alcoholic)

1. Dehydrate tissue as usual through an ascending series of aqueous ethanol solutions to absolute anhydrous ethanol.

2. Pass tissue through an ascending series of ethanolic GMA solutions (20, 40, 60 and 80 GMA in anhydrous ethanol) --30 minutes each. Then go to step #3 below. Delicate tissue should be exposed to GMA gradually over a period of several days by passing through the GMA-alcohol series at the rate of 2 changes per day. More rugged tissue may be transferred directly from 100 percent anhydrous ethanol to 100 percent GMA. In any case, the tissue is placed in 100 percent GMA for at least 12 hours.

3. Immerse in 100 percent GMA (unpolymerized)--12 hours or longer.

4. Place the infiltrated tissue and a drop or two of pre-polymerized GMA in a size 00 gelatin capsule, and orient as desired. Fill the capsules TO THE TOP with pre-polymerized GMA, insert identification tag-and-cap. Oxygen inhibits polymerization. If air bubbles are seen in the GMA, allow the capsules to remain at room temperature for 30 minutes prior to polymerization to allow the air bubbles to rise from around the tissue.

5. Place the capsules in capsule holders and place in ultraviolet apparatus until polymerized (12-24 hours). (Cole, 1968).

The temperature within the UV polymerization apparatus will rise above ambient during polymerization. If very heat-labile cell components are to be preserved, place the polymerization apparatus in a freezer at -16°C. At an ambient temperature of -16°C, the temperature of the GMA will be about 2 to 4°C. An alternate to UV polymerization is to use heat. Polymerize the plastic by placing the capsules at 40°C for 24 hours, then at 60°C until the plastic is hard (2 days or longer). The plastic may be considered polymerized when the upper surface of the plastic appears whitish, or when the surface cannot be dented with your thumbnail.

6. The gelatin capsule may be peeled off after it has been softened by soaking in water.

7. Polymerized blocks may be stored at room temperature indefinitely.

8.**TRIMMING:**
Carefully trim the block with a clean, dry razor blade. The block may be held in a collet-type holder and observed with a dissecting microscope for ease of trimming. The block face should be small (2 x 2mm) in cross-section for optimal sectioning.

9.**SECTIONING:**
Section with a dry glass knife (or diamond knife if hard tissue is present) on an ultramicrotome or with a glass or steel knife on a standard rotary microtome. Since polymerized GMA is much harder than paraffin, if a steel knife is used, it must be re-honed often. Sections are generally cut 0.5 to 2 microns thick. Because of static electricity, dry sections are sometimes awkward to handle.

Each section can be lifted from the knife edge with fine-pointed watchmaker's forceps or small brush and placed on a drop of distilled water on a microscope slide. Slides

should be cleaned, but should not be subbed or coated with albumin. The water is allowed to evaporate, and the sections are stained the next day. As a faster alternative the water may be drawn away with absorbant paper and air dried. The sections may then be stained immediately.

GMA is humidity sensitive. If blocks are too hard to section, place them in a humid atmosphere for 1-2 hrs. Conversely, if the blocks are too soft, place them in a desiccator for 1-2 hrs.

If combined LM and TEM studies are to be carried out employing the same embeddment, it is recommended that the TEM formulation be used, since it is easier to cut thick sections from the TEM blocks than to cut thin sections from the blocks prepared for LM.

10. **GENERAL STAINING PROCEDURE:**

Conventional staining procedures are used, with a few slight modifications. The plastic does not have to be removed. Place the dry slide in dye solution or place a single drop of dye solution on the section. The time of staining may be prolonged to increase intensity of staining. Sections adhere tightly to the glass and may be rinsed under a stream of water. Sections sometimes drop off or become folded in alcoholic or alkaline solutions and for this reason these solutions should be avoided. Repeated wetting and drying usually does not damage the sections. After staining, slides are rinsed in water, air dried, and a drop of Euparol or other mountant is placed on the section, and a cover glass applied.

REFERENCES:

1. Bennett, H. S. et al. (1976) Science and art in preparing tissues embedded in plastic for light microscopy, with special reference to, glycol methacrylate, glass knives and simple stains. Stain Techol. 51(2):71-97.
2. Cole, M.B., Jr. (1968) A simple apparatus for ultraviolet polymerization of water soluble embedding media employed in electron microscopy. J. Microscopic 7(3):441-444.
3. Cole, M.B., Jr. and J. Ellenger (1981) Glycol methacrylate in light microscopy: Nucleic acid cytochemistry. J. Microsc. (Oxford) 123:75-88.
4. Cole, M.B., Jr. and S.M. Sykes (1974) Glycol methacrylate in light microscopy: A routine method for embedding and sectioning animal tissues. Stain Technol. 49(6) :387-400.
5. Feder, N. and T.O. O'Brien (1968) Plant microtechnique: Some principles and new methods. Amer. J. Botany 55(1):123-142.
6. Feder, N. (1970) Notes from a Short Course in Histochemistry. Vanderbilt University.
7. Kushida, H. (1970) A new method for embedding with 2-hy droxypropyl methacrylate, J. Elec. Micrs. 19(3):281-282.
8. Leduc, E.H. and W. Bernard (1967) Recent modifications of the glycol methacrylate embedding procedure. J. Ultrastruct. Res. 19:196-199.
9. Ruddell, C. L. (1971) Embedding media for 1-2 micron sectioning. 3. Hydroxyethyl methacrylate-benzoyl peroxide activated by pyridine. Stain Technol. 42(2):77-83.
10. Cole, M.B., Jr. (1982) Glycol methacrylate embedding of bone and cartilage for light microscopic staining. J. Microscopy. (In Press).

DISCLAIMER: