SPI Chem^{TM} GMA Kit “Low Acid”

Use Instructions

SPI-Chem™ Low-Acid GMA Water Soluble Embedding Kit for Transmission Electron Microscopy (TEM) Applications

Instructions for Use of SPI #02630-AB and SPI #02630-AA

CAUTION:

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

Introduction:

The polar water soluble, 2-hydroxyethyl methacrylate, commonly known as "glycol methacrylate", or GMA, has found an increasing number of applications for the embedding of biological tissue for transmission electron microscopy (TEM), for the preservation and observation of fine structure not previously subjected to solvent dehydration. GMA forms only non-crosslinked straight chains on polymerization and therefore requires no hardener. The catalyst used to promote end-to-end polymerization is benzoyl peroxide, at a concentration of about 0.6% in the final infiltrating medium.

GMA embedments, and specifically low acid GMA embedments, offer a number of advantages over other systems:

- Dehydration of tissues can be made directly in aqueous dilutions of GMA or optionally in organic solvents
- GMA does not need to be water-free and indeed it works best with at least some water present.
- Infiltration of tissue with monomer can be performed at room temperature or lower.
- Polymerization of GMA can be performed at ambient temperature of 0°C with UV radiation to 60°C in an oven.
- Thin sections of polymerized GMA can be cut with glass or diamond knives.
- Sections from Low Acid GMA, unlike ordinary technical grade GMA, resist the uptake of stain, thereby reducing greatly the occurrence of non-specific background staining.
- Enzyme digestion, a variety of stains and immunological localizations may be performed on thick sections of GMA without removal of the plastic.

The Low Acid GMA Kit for TEM consists of three components, that is, glycol methacrylate monomer (low acid), a polymerization initiator or catalyst (benzoyl peroxide) and a plasticizer or filler (butyl methacrylate). The GMA monomer is supplied with methacrylic acid substantially removed and with small amounts of inhibitors present, to inhibit spontaneous polymerization during synthesis and storage. This inhibitor need not be removed before use. The benzoyl peroxide catalyst as supplied is provided in granular form.

Formulations:

The GMA embedding mixture for use with TEM applications is a modification of the formula of Leduc[1] (1967) which is used by Cole[2] (1981):

Recommended Amounts of GMA Components for TEM Applications

Component: Standard Formula for Final Medium

GMA (Low Acid): 67 ml
Water: 3 ml
Butyl Methacrylate: 30 ml
Benzoyl Peroxide: 0.60g

In the following preparative procedures, the 85% and 98% aqueous dilutions are made from the above, as is the pre-polymerized GMA. The solubility of GMA in water may be increased by reducing the temperature to 4-8°C. The complete, final medium, as shown above, can be pre-polymerized.

Special comments about storage of the benzoyl peroxide: Avoid loss of water content from the benzoyl peroxide as the dry material is considerable more hazardous and will burn vigorously. Store below 38°C (100°F) to maintain product's activity.

Special safety comments about GMA use: The liquid GMA component may cause severe contact dermatitis or allergic reactions in some individuals. Latex surgical gloves seem to help prevent the contact dermatitis. The polymerized GMA block seems to be non-reactive and non-toxic.

Mixing of the Resin Components:
The preparation and mixing of the resin components must be thorough and performed in a fume hood. The mixture is stored in a brown glass bottle in a freezer and is stable for 6 months or longer. Prior to use, allow the mixture to reach room temperature before opening the container, to prevent condensation from contaminating the solution.

Special Precautions:
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.

The liquid GMA components may cause contact dermatitis or allergic reactions in some individuals. Latex surgical gloves seem to help prevent the dermatitis. The polymerized GMA appears non-toxic.

Dissolve the benzoyl peroxide in the water-glycol methacrylate-butyl methacrylate mixture. Mix well and then pour enough of the solution into a 125ml or 250ml Erlenmeyer flask (Pyrex) to form a shallow (5 to 7mm) 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.

Dehydration, Infiltration and embedding

Small specimens ($1mm^3$) or samples of tissue of a size commonly used in TEM may be dehydrated in aqueous dilutions of glycol methacrylate. Adequate tissue preservation is not maintained if large samples are dehydrated with GMA. After rinsing of the fixed* tissue in buffer or water, specimens or tissue samples are transferred directly to 80% GMA in water. A typical schedule for GMA dehydration, infiltration and embedding is as follows:

Rinse	5 min. to 1 hr.
85% GMA	2 changes, 1 hr. ea.
97% GMA	2 changes, 1 hr. ea.
Final GMA embedding mixture	Overnight (not pre-polymerized)

For final embedding:

Place the infiltrated tissue and a drop or two of pre-polymerized GMA in a Size 00 gelatin capsule, orient the tissue as desired, insert identification tag, fill the capsules *to the top* or to form a positive meniscus with pre-polymerized GMA, and cap. Oxygen inhibits polymerization.

Place the capsules upright in the capsule holders and place in ultraviolet apparatus equipped with 2 long wavelength (3150A) UV lamps (G.E. #F6T5,BL) until polymerized (12-24 hours). In the presence of air or higher water content, 2-3 days may be required to complete polymerization.

*Fixing with OsO_4 should be avoided. See ref. #4.

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.

The gelatin capsule may be peeled off after it has been softened in water. Wait at least 24 hours to section if gelatin was removed after soaking in water. 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. Polymerized blocks may be stored at room temperature indefinitely.

Thin Sectioning:
When thin sectioning, the GMA cutting characteristics differ considerably from epoxy type, non-water soluble resins. Best results are obtained when the water level in the knife boat is kept to a minimum; just high enough to wet the knife edge. It has also been observed that GMA cuts better when the relative humidity is 50% or less. GMA also sections easier at higher cutting speeds and a more acute knife angle than epoxy resins.

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.

References:
[1]Leduc, E.H. and Bernhard, W., J. Ultrastruct. Res. 19, 196 (1967).

[2]Cole, M.B., Jr. Personal Communication, 1981.

[3]Cole, M.B., Jr. J. Microscopie (Paris) 7, 441 (1968)

[4]Barsotti, P. et al. J. Submicrosc. Cytol. 12, 233 (1980)

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