GLYCOL METHACRYLATE (GMA) EMBEDDING MEDIUM (WATER SOLUBLE) *(revised 7-28-00)*

Cat. No. 18350

The following improved method for GMA embedding has been described by Leduc & Bernhard, (1967). This method provides good preservation of tissues and is useful for enzymatic extraction and autoradiographic studies:

EMBEDDING MIXTURE:

Mixture of 97% GMA plus 3% distilled water	7 parts	or	70.0 ml
Mixture of 98% butyl methacrylate plus 2% Benzoyl peroxide (Paste)	3 parts	or	29.4 ml BMA 0.6 g Benzoyl Peroxide Paste

To minimize swelling artifacts, the above mixture is partially polymerized before use as follows (it should be of the consistency of maple syrup):

1. **CAUTION:**
   Use extreme care when heating such a mixture due to the risk of severe burns if the hot mixture erupts from the vessel and contacts unprotected skin. Always point the flask away from anyone nearby including yourself. Also, this step must be performed rapidly over the flame of a Bunsen burner or a torch in order to avoid premature curing of the medium if a lower temperature heat source is used over a period of several minutes.

2. Place the desired amount of the above mixture in a large, capped Erlenmeyer flask, heating over a Bunsen burner with very rapid swirling until boiling. This takes about 1 minute.

3. As soon as boiling occurs, immediately plunge the flask into a bath of ice water, and agitate vigorously until the medium cools to about 2°C. The partially polymerized mixture should have the viscosity of thick syrup. If the initial viscosity is too low, the heating and cooling process is repeated until the correct viscosity is obtained. The entire process takes about 5 minutes and the prepolymer can be stored in the freezer indefinitely.

TISSUE PREPARATION:

1. Fix in 1.25% to 2.5% Glutaraldehyde in 0.1 M Sodium Cacodylate or Phosphate buffer, pH 7.2, for 1 hour.

2. Rinse in the same buffer for 1 hour or overnight.

3. Dehydrate and infiltrate in:

   a. 80% of GMA monomer and 20% distilled water. — 15 minutes

   b. 100% 4 changes at 15 minutes each change. — 1 hour

   c. Embedding medium + Catalyst. — 1 hour

   d. Same as c.

   e. Final infiltration in partially polymerized embedding mixture. — Overnight

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NOTE: All fixation and infiltration, embedding and final polymerization with ultraviolet light are carried out in a cold room at 3°C or maintained as near as possible to that temperature with crushed ice.

EMBEDDING:

Tissue is placed in gelatin capsules (NOT POLYETHYLENE), filled to the top with fresh prepolymer. Leave capsules open for 30 minutes to eliminate air bubbles. Capsules should then be closed, leaving as little air as possible, as O_2 in air inhibits polymerization.

POLYMERIZATION:

Capsules should be held upright in supports which permit the maximum passage of UV light. With long wavelength ultraviolet light (>315 nm), polymerization takes from 25 to 48 hours, depending upon the viscosity of the prepolymer, the amount of accelerator added, and the source of UV light.

Sections should be picked up only on coated grids which can be stained later with Uranyl or Lead Acetate. Tissues embedded in GMA reveal very dense, nucleic acid-containing structures.

Glycol Methacrylate is also useful as an embedding medium for tissue sectioning for light microscopy, Hayat (1970). To process the specimens for biopsies, sections of 1 to 2µm can be cut with conventional microtomes and steel knives and stained with a variety of special stains.

REFERENCES:

Hayat, M.A. (1970). Principles and Techniques of Electron Microscopy, Vol. 2, Von Nostrand Reinghold Co., New York.

Leduc, E. & Bernhard, W. (1967). Recent modification of Glycol Methacrylate embedding procedure. Ultrastruc. Res. 4, pp. 196-199.

Rosenberg, M., Part, L.P., and Lesko, Jr. (1960). Water soluble Methacrylate as an embedding medium for the preparation of ultra thin sections. Ultrastruc. Res. 4, p. 298.

CAUTION All methacrylates (acrylics) should be considered hazardous. Direct contact and inhalation should be strongly avoided. While moderately toxic and allergenic, high concentrations may be very harmful to tissue. In addition, the methacrylates are combustible and vapors may be explosive.

All acrylics should be stored in completely airtight vessels. Experiments involving the methacrylates should be conducted only under chemical fume hoods and the user should wear the appropriate protective clothing including gloves, barrier creams, safety goggles and film forming wound sprays where necessary. Order of preference of glove material appears to be polyethylene over latex and latex over vinyl gloves. In addition, 4H gloves from Safety 4A/S in Lyngby, Denmark provide apparently superior protection over all of the standard glove materials. Latex gloves are preferred over vinyl since they fit more snugly around the fingers and are thicker, making them less vulnerable to mechanical damage.

Waste materials containing methacrylates should be stored in an airtight container in the refrigerator. Every effort should be taken to assure that the storage cabinet, including refrigerators, are sparkproof so as to avoid accidental ignition of stray acrylic vapors. In the event of direct contact with the skin, the affected area should be immediately wiped dry with clean, dry paper towels followed by a thorough washing with soap and water. Never use an organic solvent to clean embedding media components from the skin.

Tobler, M and Freiburghaus, AV, Occupational Risks of (Meth) acrylate Compounds in Embedding Media for Electron Microscopy, J. Microscopy. Vol 160, Pt 3, pp 291-298, Dec 1990.

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