Technical Data Sheet

PEG - GMA Embedding Medium

#14250

Polyethylene Glycol - Glycol Methacrylate Mix

A water - miscible embedding media is ideal for the preparation of tissues for cyto-chemical studies and enzyme localization when a correlation between light microscopy and electron microscopy is necessary, i.e., to examine a thick and a thin sections from the same block.

Embedding techniques have been improved by:

- Simplifying the method of polymerization to ensure more consistent results;
- Increasing stability under the electron beam by adding a cross-linking agent;
- Obtaining softer blocks by the addition of a plasticizing agent and thus being able to cut 1-2 micron thick sections for high resolution light microscopy;
- Improving morphological preservation by a better dehydration schedule.

Embedding Mixtures

Two embedding mixtures are recommended by SPAUR and MORIARTY (1977).

I. Modified, water-containing Glycol Methacrylate Embedding Mixture (aqueous GMA)

Glycol Methacrylate 95% GMA (7 parts)	66.5 ml.
Distilled Water	3.5 ml.
n-Butyl Methacrylate (3 parts)	28.5 ml.
Ethylene Dimethacrylate (5% v/v)	5.0 ml.
Benzoyl Peroxide (1.5% w/v)	1.5 gm.

II. Modified Glycol Methacrylate Embedding Mixture - containing Polyethylene Glycol as plasticizing agent. PEG -GMA

Glycol Methacrylate 100% GMA (7 parts)	66.5 ml.
n-Butyl Methacrylate (3 parts)	28.5 ml.
Ethylene Dimethacrylate (5% v/v)	5.0 ml.
Benzoyl Peroxide (1.5% w/v)	1.5 gm.

Polyethylene Glycol 400 (1% v/v)	1.0 ml.

Prepolymer Formation

The following materials are needed: a narrow-mouth, 125 ml Erlenmeyer flask; a #5 stopper with two holes, one at an angle to accommodate a thermometer; a short-bulb thermometer; a magnetic-stirrer hot-plate; and a dry ice-ethanol bath in 1000 ml. beaker.

Prepolymerization Procedure

- The mixture is heated to 40-45°C. on a magnetic-stirrer hot-plate with continuous stirring until the benzoyl peroxide is dissolved. Remove from heat.
- Transfer aliquots of 50 ml of the mixture to a 125 ml Erlenmeyer flask containing a "PTFE"-coated magnet. Stopper the flask with the rubber stopper, and insert the thermometer. The thermometer should be covered by the liquid, but should allow room for movement of the magnet. The other hole in the stopper is to allow ventilation.
- The flask is heated on the hot plate at a rate of 6-7°C./min. while being constantly stirred until it reaches 98°C.*
- Immediately after 98°C. is reached, remove the flask from the heat and plunge it into the dry-ice-ethanol bath with rapid swirling to flash cool the mixture to 20°C. The prepolymer can be kept in the freezer almost indefinitely.

* The 98°C. temperature is only suggested. It may vary 1 - 2°C. less if the polymerization reaction is strong, and one degree less will give the prepolymer a good viscosity, i.e., a thick syrup at 0 - 4°C.

Dehydration

Dehydration and infiltration can be done at room temperature, and also can be done at 4°C. on ice, with the time extended for each step.

Modified Dehydration Schedule - complete with glycol methacrylate monomer - GMA

80% GMA in distilled water	2 changes 10 minutes each
95% GMA in distilled water	10 minutes
100% GMA	3 changes 10 minutes each
Anhydrous unprepolymerized mixture	15 minutes
Prepolymerized mixture	24 hours

Dilutions of GMA are made with distilled water. The anhydrous mixture is made in the same proportions of the embedding mixture except without water and 100% GMA. (See Mixture I.) This procedure insures a complete dehydration and a good infiltration with results comparable to those achieved using Araldite 6005.

Embedding

The tissue is embedding in gelatin capsules (not polyethylene) with a fresh prepolymer mixture. Leave the capsules open for 30 minutes to eliminate air bubbles, or for 10 minutes in a vacuum chamber, then cap with as little air as possible trapped within the capsule. The polymerization is accomplished by placing the capsule 10 - 20 mm under a long wavelength, ultraviolet light source (3150 W) for 12 to 16 hours, depending on the block, it can be accomplished by irradiation of the top for 1 - 2 hours. Bubble formation is a occurrence and causes no normal problems.

The gelatin capsules can be removed after polymerization by soaking in warm water until all gelatin is dissolved.

Sections can be collected on coated or bare copper grids (200 mesh), and stained by routine methods with Uranyl acetate and Lead citrate.

Light Microscopy

Glycol methacrylate (GMA) is a useful embedding medium for sections for High Resolution Light Microscopy. Sections of 1 to 1 micron can be cut with a conventional rotary microtome and steel knife, or glass Ralph Knives, and stained with a variety of special stains. This technique is of special use for biopsies when a light and electron microscope are needed.

References

1. Luduc and Bernhard, W. Recent modification of glycol methacrylate embedding procedure. Ultrastructure Res. 4 p. 196 - 199 (1967).
2. Rosenberg, M., Part, L.P., Lesco, Jr. Water soluble methacrylate as embedding media for the preparation of thin sections. Ultrastructure Res. 4 p. 298 (1960).
3. Spaur, R. C. & Moriarty, G. Improvements of glycol methacrylate I. Its use as an embedding medium for electron microscopy studies. J. Histochem. Cytochem 25 : 163 (1977).
4. Swartz, W.J. & Nusbickel, F.R. Histological investigation of glycol methacrylate embedded chick embryonic tissue. J. Microscopy 115 : 101 (1979).
5. Formulas and recommendations taken from Dr. Spaur's paper .