Technical Data Sheet

Glycol Methacrylate GMA Water Soluble Embedding Medium

#14200

Introduction

Glycol Methacrylate (GMA) was introduced as an embedding medium for ultrastructural cytochemical studies in Electron Microscopy.

An improved method for GMA embedding was described by Leduc & Bernhard(1967). This method provides a better preservation of the tissues and was more useful for enzymatic extraction and autoradiographic studies. The following is the procedure which was recommended by Leduc & Bernhard.

Recommended Procedure

Fixation

- Tissue should be fixed in an 1.25% Glutaraldehyde in 0.1M Sodium Cacodylate or Phosphate buffer, pH 7.2, for 1 hour.
- The tissue should be rinsed in the same buffer for 1 hour or overnight.

Dehydration & Infiltration

- 80% of GMA monomer and 20% distilled water for 15 minutes
- 100% GMA- 4 changes at 15 minutes each change
- Embedding medium+catalyst for 1 hour.
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- Final infiltration in partially polymerized embedding medium overnight.
  Note: All fixation and infiltration, embedding and final polymerization with UV light are carried out in a cold room at 3°C or on top of ice.

Mixing Instructions

- Mixture of 97% GMA plus 3% distilled water
- Mixture of 98% butyl methacrylate plus 2% 2,4 dichlorobenzoyl peroxide(Luperco)

Final Mixture

- 7 parts or 70ml of mixture 1
- 3 parts or 29.4ml BMA of Mixture 2 and 0.6g Luperco

To reduce the swelling of artifacts, the above mixture is partially polymerized before use. It should be of the consistency of maple syrup.

The prepolymer is prepared as follows:

Place a small amount of the above mixture in a large, capped flask, while heating over a bunsen burner with very rapid swirling until it boils. This should take approximately 1 minute.

The flask is plunged immediately into a bath of ice water and agitated vigorously until it cools to about 2°C. If the initial viscosity is lower than that of the consistency of maple syrup, the heating and cooling process should be repeated for several times. The entire process takes about 5 minutes and the prepolymer may be stored in the freezer indefinitely.

Embedding

The tissue is placed in gelatin capsules (NOT POLYETHYLENE), filled to the top with fresh prepolymer. Capsules should be closed, leaving as little air as possible.

Capsules should be held upright in supports which permit the maximum passage of UV light. With long wave UV light polymerization takes from 25-48 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 with uranyl acetate or lead acetate. Tissues embedded in GMA have very dense, nucleic acid containing structures.

GMA is also useful as an embedding medium for sectioning of tissue for light microscopy (1-2 microns).

References

Leduc, E. & Bernhard, W.(1967), Ultrastructure Research 4, 196-199.
Rosenberg , M., Part, L.P., and Lesko, JR.(1960), Ultrastructure Research 4, 298.