Methacrylate Embedding Media

#E14520

(Methyl Methacrylate/Butyl Methacrylate, MBM)

Methacrylate embedding media consists of a mixture of n-Butyl and Methyl methacrylate with benzoyl peroxide as the catalyst. (Benzoyl peroxide is supplied as a paste, a mixture of benzoyl peroxide powder with dibutyl phthalate 1:1 w/v.) The desired hardness of the block can be obtained by adjusting the relative proportion of n-butyl and methyl methacrylate; a higher proportion of methyl methacrylate will produce harder blocks. Relatively soft blocks are good for soft tissues, such as pancreas, while harder blocks are suited for tough tissues such as tendon, muscle, and plant. The hardness of the blocks is obtained by mixing n-butyl and methyl methacrylate in the ratio of 4:1; 3:1; 3:2...(v/v) respectively. The ratio of 4:1 is the most commonly used. The addition of initiator, benzoyl peroxide paste, is at a ratio of 1.5-2% of the total volume of the mixture.

Methacrylate embedding may be used for embedding mineralized or unmineralized specimens. Because of the large shrinkage that occurs during polymerization of methacrylates, direct embedding is not recommended for most biological tissues, and it is therefore partially polymerized methacrylate (or pre-polymerized) which is required.

Using Methacrylate Embedding Media

1. Preparation of Pre-polymerized MBM:
It is not necessary to remove polymerization inhibitors from the methacrylates. The mixture of methyl and butyl methacrylate plus catalyst (MBM) is prepared by mixing n-butyl and methyl methacrylate per the ratio that you chose with the addition of 1.5-2% benzoyl peroxide paste. Mix well, then place the mixture in a water bath at 65°C until the temperature of the solution slightly exceeds 65°C. The reaction temperature of the solution should be maintained between 65 and 70°C until the viscosity of the liquid resembles that of room temperature glycerine (about 30-40 minutes). The reaction temperature is not allowed to exceed 70°C. The solution then is cooled in an ice bath and stored in a refrigerator. This pre-polymerized stock solution is stable for about one year when kept refrigerated.

2. Preparation of Methacrylate Catalyst Stock Solution:
The methacrylate catalyst stock solution consists of 2 g benzoyl peroxide paste and 18 g methyl methacrylate monomer. Mix well. The catalyst stock solution is then stored under refrigeration.

3. Embedding mixture:
Every 1 ml of catalyst stock solution is mixed well with 5 ml of prepolymerized MBM.

4. Embedding Schedules:
Methacrylates are readily soluble in ethanol and acetone and no intermediate solvent is required. The standard embedding schedule is:

Ethanol/MBM 1 hour

MBM 1 hour

MBM 1 hour

Place specimens in gelatin or polyethylene embedding capsules and fill the capsules with the embedding mixture. Close the capsule with their caps. Polymerization takes place over night at 60-65°C or 24-48 hours under UV light.

5. Sectioning and Mounting:
The sections should be cut on a dry knife (Diamond or Glass Knife) 1 to 3 microns in thickness. Sections can be picked up and floated on a drop of water on a glass slide that has been treated with a thin coating of an albumin/glycerin (1:1, v/v). The slide is then placed on a hot plate and heated for a few seconds to dry the water drop and to adhere the section to slide. Prior to image analysis or staining, contrast is enhanced by immersing the slide in 2-butanone to remove the embedding medium.

References:

1. A.M. Glauert (1975). Practical Methods in Electron Microscopy. North-Holland Publishing Company.
2. M.A. Hayat (1989). Principal and Technique of Electron Microscopy. 3rd Edition, CRC Press, Inc.
3. E.K. Boylston et al., (1995). A quick Embedding Method for Light Microscopy and Image Analysis of Cotton Fibers. Biotech. & Histochem. pp. 24-27.