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HEMA (GMA) Embedding Kit Instructions

Catalog Number: 70470

Contents: 450ml Hydroxyethyl Methacrylate
100ml Butoxyethanol
50ml PEG/Dimethylaniline
10g Dibenzoyl Peroxide

1. Fixation and Dehydration

- We recommend aqueous buffered glutaraldehyde and/or formaldehyde solutions (Catalog Numbers: 201xx, 202xx, 20295 or 20300).
- Since HEMA is miscible in water, many users carry dehydration only to the 95% ethanol stage and then transfer the specimen to the infiltration medium (see below).
- Methyl Cellosolve (Catalog Number 70320) is also recommended as a dehydrating agent.

2. Infiltration

- The standard infiltration medium contains: 8ml HEMA, 1ml butoxyethanol and 0.1g dibenzoyl peroxide.
- This mixture is stirred or shaken at room temperature until the catalyst has dissolved completely.
- We recommend two changes of the infiltration medium and a total infiltration time of 9-12 hours.

3. Embedding

Since oxygen inhibits the polymerization of HEMA, embedding must be carried out either in a closed container such as a BEEM capsule (Catalog Numbers 21600-21607) or in a plastic embedding mold in an oxygen-free atmosphere.

The polymerization accelerator contains dimethyl aniline in polyethylene glycol and is premixed. One volume of this mixture is added to 25 volumes of the infiltration medium, and the infiltrated tissue is transferred to this mixture. Polymerization then proceeds at room temperature for at least 2 hours but preferably overnight.

4. **Sectioning**

Sections can be cut on a rotary microtome with either a steel or a glass knife. If a steel knife is used, it may be necessary to reduce the block hardness by increasing the proportion of butoxy ethanol in the embedding medium. Allow blocks to equilibrate with atmospheric humidity before sectioning. Freshly polymerized blocks may be relatively brittle before this equilibrium is complete.

Glass knives of the Ralph-Bennett type are particularly recommended for sectioning this plastic. Our glass knife kit (Catalog Number 70830) contains all the components and instructions necessary for making these knives.

Individual sections are removed from the knife edge with tweezers (e.g., #10610) or with a sable brush (e.g., #23200) and are flattened on the surface of a drop of water on a cover slip or slide.

No mountant is needed at this stage. The sections will adhere strongly to the glass surface when dry.

5. **Staining**

The hydrophilic nature of the plastic matrix allows most conventional water-soluble stains to penetrate and bind to the embedded tissue. Few modifications of standard techniques are required.

6. **Variations**

- The overall block hardness can be controlled by varying the percentage of butoxy ethanol within the range of 7-14% (vol/vol). The standard recipe given above contains 11.1% butoxy ethanol and produces blocks of medium hardness, adequate for most purposes.
- Block hardness is determined not only by the butoxy ethanol concentration but also by the rate of polymerization and the amount of the secondary plasticizer, polyethylene glycol.

The standard recipe uses a 25:1 ratio of infiltration medium to accelerator. This ratio (which corresponds to a roughly two-fold molar excess of dimethylaniline to benzoyl peroxide) produces quite rapid polymerization (~30 minutes). Harder blocks and somewhat slower polymerization result when this ratio is raised to 50:1.

- The investigator is urged to consult the original literature for conditions best suited to a particular application.

7. Storage of Kit Components

- HEMA should be stored in a dark glass bottle at 4°C.
- Butoxy ethanol requires no special storage precautions.
- Benzoyl peroxide should be stored at 4°C.
- The complete infiltration medium containing HEMA, butoxy ethanol and benzoyl peroxide can be stored in the dark at 4°C for up to 10 days. Longer storage times are not recommended.
- The PEG/dimethyl aniline accelerator is stable at room temperature for several months.

8. Handling

HEMA, like all acrylic esters, should be handled with caution. Avoid contact with the liquid and vapor phases of this chemical. We recommend the use of disposable gloves and a chemical fume hood when working with this embedding medium.

The cured resin is quite safe to handle but we recommend that any residual unpolymerized material on the surface of a block be trimmed off with a razor blade before the block is handled without gloves.