Technical Data Sheet

Micro-Bed Embedding Resin

#14210

Micro-Bed resin is a water-soluble resin which is a mixture of methacrylate and polyester resins. It has been developed specifically for the study of animal, plant, and microbiological samples, in both light and electron microscopy.

Tissue Processing

Small pieces of tissue (<1mm) are processed in 1/2 dram shell vials (EMS Cat.#72630-05) as described below:

1. Fix in a mixture of formaldehyde (4%), glutaraldehyde (1%) in sodium cacodylate buffer (0.1M, pH 7.4) for 3 hours at 4°C. (Avoid osmium tetroxide and coloring fixations, such as potassium permanganese, picric acid etc. Use tannic acid as an alternative if needed).
2. Wash thoroughly in buffer (three changes). Sodium cacodylate buffer is recommended.
3. Dehydrate in a graded ethanol or acetone series (70%, 90%, and 100%), 10 minutes each.
4. Infiltrate with 100% Micro-bed resin, 3 changes, 40 minutes each at room temperature.
5. Infiltrate with fresh resin for 8 to 10 hours or overnight, gently agitating prior to final polymerization.

Embedding Schedule

1. **Thermal Cure:**
   From step 1 above, tissues can be embedded in embedding capsules (EMS Cat. #70000), gelatin capsules (Cat. #70100) or flat embedding molds (Cat. #70900) with fresh Micro-bed resin at 50-60°C in an oven for 24-48 hours. (The exclusion of oxygen during polymerization is not necessary).
2. **UV Light Cure**:
   From step 5 above, tissues can be embedded in embedding capsules or gelatin capsules (EMS Cat. #70100) and polymerized with UV irradiation at any temperature from -10°C to 20 °C for 48 to 72 hours.
   During polymerization, which is an exothermic reaction, some heat will be generated. It has been suggested that a cooler environment will reduce the temperature of the tissue and preserve its antigenicity. In this case, a tray filled with ice could be used as an embedding tray.
3. **Low Temperature Cure or Progressive Lowering of Temperature:**
   Micro-bed will remain in its liquid form down to -50°C. If the specimens are particularly sensitive to solvents (such as alcohol) at ambient temperatures, it is recommended to dehydrate the tissue in a graded alcohol series while progressively lowering the temperature. Then, step 3 above should be as follows:

   Dehydrate in a graded alcohol series: 30, 50, 70, 95 and 100%, while reducing the temperature according to each stage, 1 hour each . Follow with 2 changes, one hour each with fresh Micro-bed resin at -30°C. Infiltrate with fresh Micro-bed resin overnight or for at least 8 hours at -30°C, periodically agitating. Polymerize with UV light at your chosen temperature for at least 70 hours.

   A control block is necessary for testing the cutting quality of the final block.

Sectioning

For LM studies, 0.5-2 micron sections are easily obtained with glass knives or diamond knives.

For EM studies, 0.1-0.2 micron sections are also easily produced with a diamond knife. For good stability in the electron beam, grids with formvar or carbon coatings are needed to pick up the sections.

Staining

For LM, a standard staining protocol can be used (hematoxylin, eosin, or toluidine blue).

For EM, the normal heavy metal counterstains(uranyl acetate) work well.

References

Scala C. Cenacchi G, Ferrari C, Pasquinelli G, Preda P, Manara GC (1992). A new acrylic resin formulation: A useful tool for histological, ultrastructural and immunocytochemical investigations. J. Histochem Cytochem , 40 (11), 1799-1804.

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Berryman MA, Porter WR, Rodewald RD, Hubbart AL (1992). Effects of tannic acid on antigenicity and membrane contrast in ultrastructural immunocytochemistry. J. Histochemistry and Cytochemistry 40, 6, 845-857.