INSTRUCTIONS

PELCO™ Acrylic Embedding Resin - Cat. No. 18190
500 ml

Introduction: PELCO™ Acrylic Resin is a mixture of 2-hydroxyethyl methacrylate, 2-hydroxypropyl methacrylate, *n*-butyl methacrylate and styrene. The catalyst, benzoyl peroxide, is supplied in a separate container. The resin is designed to give good ultrastructural preservation and be appropriate for immunocytochemical and histological staining. It is a hydrophilic resin which can be polymerized by either heat or UV (~365nm) light.

Storage: The uncatalyzed resin can be stored at room temperature. It is stable under these conditions for at least two years. For best storage the resin should remain uncatalyzed. It is best to add catalyst only to the amount of resin you intend to use at any one time. After addition of the catalyst the resin is best kept at 4°C or colder. Periods of up to 24 hours at 20°C or less are all right during room temperature infiltration steps.

Mixing: The catalyst is added to the resin at 1.3% w/v (1.3g/100ml). Mix gently on a magnetic stirrer until the catalyst has completely dissolved. Store at -20°C until ready to use. The resin will remain liquid at temperatures down to -50°C.

Handling: All handling of the resin should be done in a fume hood with gloves and preferably goggles as well. The aroma is quite pungent and the breathing of vapors should be avoided. All waste containers, towels etc. should be polymerized prior to disposal.

Dehydration: Ethanol or acetone can be used as a solvent. A 50-70-90-100% or 70-90-100% dehydration series can be employed. For steps below 100% 2 x 10min. per step is recommended. For the 100% step 3 x 10min. is recommended.

Infiltration: A 1:1 mixture of solvent:resin can be employed for a time period appropriate to the tissue being processed. This period can be 1 to 2 hours or overnight depending on the tissue. This step should then be followed by 3 changes in 100% resin. If the 1:1 step is overnight then the 3 100% changes can be short (i.e. 3 x 1hr.). If a short 1:1 step is employed then it is advisable to have the last 100% step be 8 hours. In this way adequate infiltration is ensured. If the tissue being processed is known to be relatively easy to infiltrate then the 1:1 step can be skipped and 3 changes in 100% resin can be used. The final step should be at least 8 hours to ensure best results.

Polymerization: Tissue samples can be embedded in either BEEM® or gelatin capsules. **To minimize shrinkage during polymerization it is best to cap the container being used for embedding during polymerization.** Flat embedding is not recommended due to damage to silicon rubber molds and resin shrinkage during polymerization. If flat embedding must be used it is best to use a Teflon mold (Cat. Nos. 10506 or 10508) covered with ACLAR® film for best results.

Oven polymerization: Temperatures from 50-60°C are recommended. At 50°C it will require from 2 to 3 days to achieve adequate polymerization for ultrathin sections. At 60°C it will require from 1 to 2 days to achieve adequate polymerization for ultrathin sections. Thick sections can usually be cut after as little as 24 hours polymerization. Temperatures above 60°C will shorten the polymerization time to ultrathin sections but will also cause the resulting blocks to be quite brittle.

UV polymerization: The use of osmium tetroxide, picric acid or other similar fixatives/stains can affect the absorption of UV light by the specimen and lead to problems during polymerization. Long wavelength (~365nm) UV light should be used. Do not use bulbs that emit both long and short wavelength light. The UV bulbs can be placed above or below the specimens. Regardless of light placement (above or below the embedding blocks) a reflective shield/substrate should be employed to insure uniform polymerization throughout the embedding capsules.

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The embedded specimens can be polymerized from temperatures ranging from 20°C down to -45°C. Optimum polymerization is believed to be achieved at 4°C using 2 each 8 watt bulbs positioned 15 cm from the sample. Under these conditions the resin will be adequately polymerized for ultrathin sectioning in 2-3 days. Too rapid a polymerization will cause bubble formation in the blocks. Temperatures below 4°C will necessitate either higher wattage UV bulbs or closer sample placement to achieve polymerization in 2-3 days. It is best to do a trial polymerization run to evaluate the parameters of the UV setup being used.

Applications:

1. Progressive lowering of temperature: Due to the low freezing point (-50°C) of the resin it can be used in protocols requiring progressive lowering of temperature during dehydration, infiltration and polymerization.

2. Light microscopy studies: Due to the unique staining properties of the resin it can be used with many polychromatic stains used in histology. The properties of the resin also make it ideal for immunohistochemical studies.

3. Electron microscopy immunocytochemistry. The hydrophilic nature of the resin and its cutting properties make it ideally suited for immunogold labeling studies.

Post staining of sections for electron microscopy: Aqueous uranyl acetate (2%) works best when staining times are around 5 minutes. A short 30 second rinse followed by staining on lead for 3-5 minutes works well.

Cutting properties: The resin is somewhat brittle after polymerization. Due to its hydrophilic nature the water level during thick or thin sectioning should be adjusted so that the block face does not wet during cutting. A cutting speed of 1mm/sec or less is recommended. Glass or diamond knives can be used.

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