JB-4 Embedding Kit

#E14270-00

JB-4 Embedding Kit is a unique polymer embedding material that gives a higher level of morphological detail than Paraffin processed tissues. A water-soluble media, JB-4 does not require dehydration to absolute alcohol except for dense, bloody, or fatty tissue specimens. JB-4 excellent for non-decalcified bone specimens, routine stains, special stains, and histochemical staining. Clearing agent such as xylene and chloroform are not required. The polymerization of JB-4 is exotheremic, which is easily controlled by polymerizing on ice or by using refrigeration at 4°C, JB-4 embedding kits must be used under a chemical fume hood.

Sections of JB-4 embedded material can be cut at 0.5 to 3.0 microns or thicker. Microtones designed for plastic sectioning are required as are glass, Ralph, or tungsten carbide, or for the best result diamond knives. Diatome U.S. has good selection of Diamond Histo-Knives, which are excellent for sectioning JB-4 block.Sections can be stained for routine histological or histochemical procedures. Immunohistochemical procedures are not recommended for JB-4 as the glycol methacrylate cannot be removed from the section and may block antigen sites for most antibody reactions. As an alternative we recommend the LR White, Unicryl or Micro-Bed (See EMS catalog for more information)

Note: It is recommended that the Embedding Kit be used under a chemical fume hood with appropriate gloves.

Fixation
Specimen can be fixed in 10% Neutral Buffered Formalin or other routine histological fixative. We suggest using HISTOCHOICE™ MB® (EMS Cat #16786), a formaldehyde-free fixation. Routine specimen sizes for soft tissue should be no more than 2 x 2 x 2cm with fixation at a minimum of four hours to overnight. Fatty or dense tissues should be fixed overnight. Lager bone specimens, will requires fixation overnight or longer depending on the specimen size. Fixation can be at room temperature or 4°C. Cold fixation will extend the time required for the specimen to be penetrated and fixed. Large bone specimens will require longer fixation times, dependent on the size and density of the bone. Decalcification is not required for JB-4 embedded specimens.

Dehydration
Dehydration can be completed at room temperature or 4°C. This process can also be done with a routine tissue processor, stopped at the end of the last alcohol step and removed for infiltration. Please note that polymers cannot be used in routine histology tissue processors, at any time. It may void the warranty and possibly begin to polymerize in the system thereby blocking the lines. Check with the manufacturer priot to attempting infiltration on any unit.

Infiltration
Infiltration Solution Mixing Procedures:

The following amounts of material are used for one 100ml batch of infiltration:

- JB-4 Solution A (Monomer) 100 ml
- Benzoyl Peroxide, Plasticized (Catalyst) 1.25 g

Carefully weight 1.25 g of catalyst (benzoyl peroxide plasticized) and add to 100 ml Solution A while stirring on a magnetic stirrer. Mix until dissolved approximately 10 to 20 minutes. Measurement of the catalyst is critical as it will control the rate of polymerization of the plastic and the exothermic reaction. This infiltration solution can be stored for up to two weeks in a dark cool area or in the refrigerator at 4°C.

Infiltration Procedure
Infiltration is performed at room temperature or at 4°C. Do not expose the samples to heat or direct light during infiltration. The specimens should be placed in two to three changes of infiltration solution to allow for the removal or replacement of all alcohols or tissue fluids.

The amount of infiltration solution used is approximately 8 to 10 times that of the volume of the specimen. The changes of fluid should be every 10 to 90 minutes for smaller specimens. The time in each change is dependent on the size of the specimen. When infiltration is complete the tissue generally appears translucent and in most cases will sink to the bottom of the container.

Infiltration should be done on s slow rotator, hematology shaker table or inverted several times during the process to allow complete saturation.

Embedding
The polymerization process should be under anaerobic conditions with the use of block holders, under light vacuum or in an air-tight container.

Prior to mixing the Embedding Solution collect andprepare the following materials; embedding molds, block holders, labels, gloves, instruments, an ice bath, and the specimens. Do not pre-cool the molds as this may cause condensation and prevent even polymerization of the block face. To prevent polymerization from occurring too fast and possible overheating of the tissue it is recommended that the polymerization process for embedding be slowed by completing it in the refrigerator or in a cold room at 4°C. Note that this may extend the polymerization from several hours to overnight.

Larger specimens with increased embedding solution may have an even greater exothermic reaction. Using a 4°C refrigerator or cold room should control this. These larger specimens will require longer times for complete polymerization and may have more unpolymerized liquid on top of the block.

Embedding Solution Mixing Procedure:
Make fresh Solution A following the directions in infiltration solution and procedures above. Do not use old or used catalysed Infiltration Solution for the embedding solutions

The following amounts of material are used for 25 ml of embedding solution:

- Infiltration Solution 25.0 ml
- JB-4 Solution B (Accelerator) 1.0 ml (must be an exact measurement)

Mix 25 ml of fresh made Infiltration Solution and 1.0 ml of JB-4 thoroughly and begin embedding immediately. The molds should be cover or place under vacuum at no more than 15psi t exclude oxygen during the polymerization process. If anaerobic conditions are not maintained, the JB-4 may polymerize incompletely or not all. Embedding capsules or gelatine capsules are recommended.

We recommended polymerization in the refrigerator at 4°C or on an ice bath to reduce the exothermic reaction to 55°C or less. Room temperature will be completed in 1 to 2 hours for smaller blocks and can go up to 3 hours or more for very large blocks. Note that the exothermic reaction can exceed 100°C for larger specimens using 10 to 50 ml of embedding solution at room temperature, therefore lage blocks should be polymerized in the refrigerator or on ice. The blocks may range in color from light yellow to dark yellow or amber. This color shift is not a problem and will not effect the block hardness. The top of the block may have a liquid on it that can be removed by draining or drying the block in a desiccator for several hours to overnight.

Deplasticizing and Staining
JB-4 is a glycol methacrylate based polymer and it cannot be removed from sections, therefore no organic solvents are required. Routine histology stains and most histochemistry can be run on the sections. High molecular weight special stains or immunohistochemical reactions may not penetrate the polymerized plastic in the sections

Warning
May be harmful if swallowed. Use under a fume hood with appropriate gloves. Components may cause irritation and or allergic skin reaction. Avoid contact with eyes, skin and clothing. Avoid inhalation of the vapors. Wash hands or expose areas thoroughly after handling the solutions.

Do not heat over an open flame. Avoid electrical or static sparks. Store un-catalysed resin in the original containers at room temperature in a dark cool place.

First Aid
In case of contact with any component or mixed solution, immediately flush area with plenty of water for at least 15 minutes. Should either unpolymerized or polymerized material contact the eyes flush with water for at least 15 minutes. If swallowed, drink excessively water and call a physician immediately. Never give anything by mouth to someone who is unconscious.

Storage
Refrigeration of the kit components is not required but they do required storage in a cool dark place. Do not store in the light or in a heated area as it may cause the monomer to polymerize. The catalyst, plasticized benzoyl peroxide, is organic peroxide that is shipped dry and does not required special storage. Please noted that the catalyst is formulated to remain stable and weigh correctly for this procedure without any adjustments to the amounts recommended. The catalyst should be kept tightly sealed. The catalyst may decompose with age, therefore we recommend carefully monitoring the date received and using the catalyst only with the kit it came in for best results.

Catalyst Disposal Procedure
The catalyst can be destroyed by slowly adding and mixing it in small portions of the catalyst at 4 times or more the volume to weight of 10% sodium hydroxide solution in water. Do not allow material to settle in lumps or stand in layers and mix until dissolved completely. Dispose of this solution, Monomer A, and the accelerator with other hazardous wastes in accordance with local, state, and federal regulations.