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TECHNICAL DATA SHEET 494

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JB-4[®] Mini Kit

INTRODUCTION:

JB-4 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 is excellent for non-decalcified bone specimens, routine stains, special stains, and histochemical staining. Clearing agents such as xylene and chloroform are not required. The polymerization of JB-4 is exothermic, 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.

The JB-4 Mini Kit is designed for use with a small number of samples or for one larger specimen. The JB-4 Mini Kit is also a convenient size for introducing the user to the advantages of the JB-4 Embedding Kit[®]. Sections of JB-4 embedded material can be cut at 0.5 to 3.0 microns or thicker. Microtomes designed for plastic sectioning are required and glass or tungsten carbide knives are recommended. Sections can be stained for routine histological or histochemical procedures. Immunohistochemistry is not recommended 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 Polysciences, Inc. Osteo-Bed Bone Embedding Kit as it is methyl methacrylate which is used for bone or for immunohistochemistry on routine histological specimens.

NOTE:

It is recommended that the Embedding Kit be used under a fume hood with appropriate gloves. For additional details, see **Warnings** and **Precautions**.

JB-4 MINI KIT CONTENTS:

Monomer A*	40mL x 2
Catalyst	0.5g x 2 in small bottles
Accelerator	2.0mL

*Please note the kit contains two bottles of Monomer A. As per the instructions, label the bottles with Infiltration and/or Embedding Solution to avoid errors in mixing and usage.

FIXATION:

Specimens can be fixed in 10% Neutral Buffered Formalin or other routine histological fixative. Poly/LEM is a methanol free formalin based fixative for light and electron microscopy developed by Polysciences, Inc. Routine specimen sizes for soft tissue should be no more than 2.0cm X 2.0cm X 2.0cm with fixation at a minimum of four hours to overnight. Fatty or dense tissues should be fixed overnight. 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 with the exact length dependent on the size and density of the bone.

DEHYDRATION:

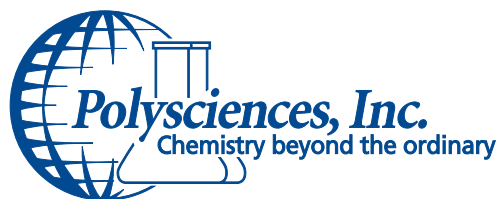
Specimens can be dehydrated in ascending grades of alcohol to 95% or absolute alcohol. JB-4 Monomer does not require complete dehydration to 100% as there is some water in the monomer. Specimens should be dehydrated for the same times as routine histological specimens. Times can range from 5 minutes to several hours depending on size and density of the specimen.

INFILTRATION:

Preparation of JB-4 Catalyzed Infiltration Solution:

Two bottles of JB-4 Monomer A are provided for ease of mixing and embedding. One bottle is used for the infiltration of the specimens only and the second is mixed and used for embedding only.

The Infiltration Solution is made by adding the entire amount of pre-measured catalyst contained in one small bottle to the 40ml bottle of Monomer A. Mix until all powder is dissolved. Label the bottle as Infiltration Solution. Unused Infiltration Solution can be stored for up to two weeks in a cool dark place or in the refrigerator at 4°C in the original bottle.



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INFILTRATION PROCEDURE:

The specimens should be covered with 8 to 10 times the volume of fluid to tissue. Specimens should be infiltrated through three changes of infiltration solution and will appear translucent and sink to the bottom when completely infiltrated. Gentle agitation or a rotator or rocker table aids infiltration. If neither of these is available invert often during the process. The infiltration times are generally the same as dehydration, however infiltration times can be lengthened if the specimen does not sink or requires longer times.

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 and prepare 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. This should be controlled by using a 4°C refrigerator or cold room. These larger specimens will require longer times for complete polymerization and may have more unpolymerized liquid on top of the block.

Preparation of JB-4 Embedding Solution:

The second bottle of Monomer A can be divided and used for two separate embedding procedures or mixed as one for a larger specimen. If the entire amount is mixed use as indicated below. Embedding Solution cannot be stored as it will begin to polymerize once the Solution B is added. If only one half of the embedding solution is required adjust the amounts as needed.

Solution A (Monomer)	40.0mL
Catalyst	0.5g (small bottle)
<i>Mix completely before adding the Accelerator</i>	
Solution B (Accelerator)	1.6mL

The embedding solution must be used immediately as it will begin polymerizing at room temperature within 30 to 40 minutes. The specimen should be placed in the bottom of the mold and all excess infiltration

solution drained off prior to orientation. The mold must be filled to above the level of the specimen. A block holder should be used to assure an anaerobic environment for the polymerizing solution. If a larger mold or form is used it must be sealed or placed in a container with a seal to avoid oxygen as this will cause the material to polymerize incompletely or not at all.

We recommend polymerization in the refrigerator at 4°C or on an ice bath to reduce the exothermic reaction to 55°C or less. Room temperature polymerization will be complete in 1 to 2 hours for smaller blocks and can go up to three hours or more for very large blocks. Note that the exothermic reaction can exceed 100°C for larger specimens using 10 to 50mL of embedding solution at room temperature. Large 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 film on it that can be removed by draining or drying the block in a desiccator for several hours to overnight.

SECTIONING:

Sectioning is done with a microtome designed for plastic sectioning. It can be done with either a glass knife, Ralph knife or tungsten carbide knife. The tungsten carbide knives can be triangular, disposable blade and holder, or any standard tungsten knife that suites your sectioning requirements. Tungsten Carbide knives are available from Polysciences, Inc.

DEPLASTICIZING AND STAINING:

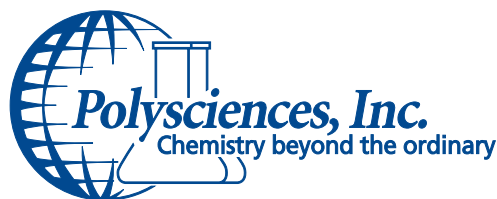
JB-4 is a glycol methacrylate based polymer and it cannot be removed from sections, therefore no organic solvents are required. Routine histology stains and most histochemistry procedures can be used. High molecular weight special stains or immunohistochemical reactions may not penetrate the polymerized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

WARNING:

May be harmful if swallowed. Use under a 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 thoroughly after handling the solutions.

PRECAUTIONS:

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



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FIRST AID:

In case of contact with any component or mixed solution immediately flush area with 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 water to excess and call a physician immediately. Never give anything by mouth to someone who is unconscious.

STORAGE:

Refrigeration of all kit components is not required but they do require 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 require special storage. Please note that the catalyst is formulated to remain stable

and weighed correctly for this procedure without any adjustments to the amounts recommended. **The catalyst should be kept tightly sealed and stored at 4°C after opening.** The catalyst may decompose with age, therefore we recommend the 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, 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.

ORDERING INFORMATION

Cat. #	Description	Size
22507	JB-4 [®] Mini Kit	1 kit
15899	Block Holders	50 holders
08379	Formalin 10% NBF	3.75L, 20L
16864	Poly/LEM Fixative (Methanol Free)	3.75L, 4 x 3.75L
16643A	Polyethylene Molding Cup Trays 6 X 12 X 5mm (20 Cavities)	1 Each
16643B	Polyethylene Molding Cup Trays 12 X 16 X 5mm (20 Cavities)	1 Each
17177A	Polyethylene Molding Cup Trays 6 X 8 X 5mm (9 Cavities) Hex	3 Each
17177B	Polyethylene Molding Cup Trays 2 X 15 X 5mm (9 Cavities)	3 Each
17177C	Polyethylene Molding Cup Trays 13 X 19 X 5mm (9 Cavities)	3 Each
24216	Tissue Tack Slides Silane Coated	72 / Box
24234	Tungsten Carbide Disposable Blades	3 / Pkg

TO ORDER

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