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TECHNICAL DATA SHEET 393

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JB-4 Plus® Embedding Kit

Catalog Number 18570

Introduction:

JB-4 Plus is a unique polymer embedding system that gives a higher level of morphological detail than paraffin processed tissues. JB-4 Plus offers all the benefits of our regular JB-4 formulation with the additional advantages of a cooler acting accelerator and the production of clear blocks. A clear polymer embedding matrix is essential to numerous histological and histochemical procedures, most notably in ophthalmic applications. A water-soluble media, JB-4 Plus does not require dehydration to absolute alcohol except for dense, bloody or fatty tissue specimens. JB-4 Plus is excellent for routine stains, special stains, and histochemical staining. *Clearing agents such as xylene and chloroform are not required.* The polymerization of JB-4 Plus is exothermic, which is easily controlled by polymerizing on ice or by using refrigeration at 4°C. All JB-4 Type Embedding Kits must be used under a chemical fume hood.

Sections of JB-4 Plus embedded material can be cut at 0.5 to 3.0 microns or thicker. Microtomes designed for plastic sectioning are required, as are glass, Ralph, or tungsten carbide knives. Polysciences, Inc. has tungsten carbide knives available for most sectioning requirements. Sections can be stained for routine histological or histochemical procedures. Immunohistochemistry is not recommended as the glycol methacrylate component 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. The Osteo-Bed formulation is a methyl methacrylate that is well suited 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**.

Fixation:

Specimens can be fixed in 10% Neutral Buffered Formalin or other routine histological fixative. We suggest using Poly/LEM, a methanol free formalin based fixative for light and electron microscopy, developed by Polysciences. Routine specimen sizes for soft tissue should be no more than 2.0cm X 2.0cm X 2.0cm with a minimum fixation time of four hours and as long as 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. Larger bone specimens will require fixation overnight or longer depending on the specimen size. Bone specimens are not decalcified prior to fixation. Note that while JB-4 Plus can be used for bone specimens, polymerized JB-4 Plus is slightly softer than the JB-4 or Osteo-Bed Bone Embedding formulations, therefore JB-4 or Osteo-Bed are recommended for most bone procedures.

Dehydration:

Dehydration can be completed at room temperature or 4°C. This process can also be done with a routine tissue processor and 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 prior to attempting infiltration on any unit.

Infiltration:

Infiltration Solution Mixing Procedure:

The following amounts of material are used for one 100ml batch of Infiltration Solution: (See **Figure 1** for different solution sizes)

JB-4 Plus Solution A (Monomer)	100.00ml
Benzoyl Peroxide, Plasticized (Catalyst)	1.25gm

Carefully weigh 1.25gm of catalyst (benzoyl peroxide, plasticized) and add to 100.00ml Solution A while stirring on a magnetic stirrer. Mix until dissolved, approximately 10 to 20 minutes. Measurement of the catalyst is critical,

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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 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 a slow rotator, hematology shaker table, or inverted several times during the process to allow complete saturation.

Figure 1: Infiltration Solution

Infiltration Solution Volume	JB-4 Plus® Solution A (Monomer)		Benzoyl Peroxide (Catalyst)
100ml	100ml	+	1.25gm
50ml	50ml	+	0.625gm
25ml	25ml	+	0.3125gm

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, on ice, or in a cold room at 4°C. Note that this may extend the polymerization from several hours to overnight.

Embedding Solution Mixing Procedure:

Make fresh Solution A following the directions in Infiltration Solution and Procedures. Do not use old or used catalyzed Infiltration Solution for the Embedding Solutions.

The following amounts of material are used for 25ml of embedding solution: (See **Figure 2** for different solution sizes)

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

Figure 2: Embedding Solution

Embedding Solution Volume	Fresh Infiltration Solution		JB-4 Plus® Solution B (Accelerator)
50ml	50ml	+	2ml
25ml	25ml	+	1ml

Mix 25ml of freshly made Infiltration Solution and 1.0ml of JB-4 Plus Solution B thoroughly and begin embedding immediately. Drain excess Infiltration Solution off the specimen before orienting it in the block holder. The small JB-4 Plus Embedding Molds from Polysciences, Inc. require approximately 1.5 to 2ml of solution per mold. The Block Holder is essential to exclude oxygen during the polymerization process. If Block Holders are not used, cover the molds with an air tight film or place under vacuum at no more than 15psi, preferably in a cold room at 4°C or a refrigerator. BEEM® capsules may be capped for embedding.

The blocks will be clear to very pale yellow. 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 and can be removed by draining or drying the block in a desiccator for several hours to overnight.

Deplasticizing and Staining:

JB-4 Plus is a glycol methacrylate based monomer formulation that cannot be removed completely 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 remaining polymerized plastic in the sections and may result in an incomplete reaction.

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

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sparks. Store un-catalyzed resin in the original containers at room temperature in a dark cool area.

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 the kit components is not required. They should be stored 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 an organic peroxide that is shipped dry and does not

require special storage. Please note 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 in small portions of the catalyst to 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:

Catalog #	Description	Size
18570-1	JB-4 Plus Embedding Kit	1 Kit
18570A-500	JB-4 Plus Solution A	500ml
18570B-15	JB-4 Plus Solution B	15ml
18570C-8	Benzoyl Peroxide, Plasticized (Catalyst)	8.0gm
16864-3.75	Poly/LEM Fixative	3.75L
16643A-1	Polyethylene Molding Cup Trays 6 X 12 X 5mm (20 Cavities)	1 Each
16643B-1	Polyethylene Molding Cup Trays 12 X 16 X 5mm (20 Cavities)	1 Each
17177A-1	Polyethylene Molding Cup Trays 6 X 8 X 5mm (9 Cavities) Hex	3 Each
17177B-1	Polyethylene Molding Cup Trays 2 X 15 X 5mm (9 Cavities)	3 Each
17177C-1	Polyethylene Molding Cup Trays 13 X 19 X 5mm (9 Cavities)	3 Each
15899-50	JB-4 Plus Plastic Block Holders	50/Pkg
24216-1	Tissue Tack Slides Silane Coated	Approx. 72/Box
24236-1	Tungsten Carbide Knife 35 Angle Wedge D	1 Each
24237-1	Tungsten Carbide Knife 45 Angle Wedge D	1 Each
24238-1	Tungsten Carbide Knife 55 Angle Wedge D	1 Each
24234-1	Tungsten Carbide Disposable Blades	2 per Pkg
24235-1	Tungsten Carbide Disposable Blades (2) and 1 Reusable Blade Holder	1 Pkg

To Order:

In The U.S. Call: 1-800-523-2575 • 215-343-6484

In Germany Call: (49) 6221-765767

In The U.S. FAX: 1-800-343-3291 • 215-343-0214

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Order online anytime at www.polysciences.com

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