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JB-4 Kit

AGR1130

Introduction

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 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.

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 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. 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 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 fixation at a minimum of four hours to overnight. Fatty or dense tissues should be fixed overnight. Larger bone specimens will require 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.

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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 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:

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

Carefully weigh 1.25gm of catalyst (benzoyl peroxide, plasticised) 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, 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.

Embedding

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

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Prior to mixing the Embedding Solution collect and prepare the following materials: embedding moulds, block holders, labels, gloves, instruments, an ice bath and the specimens. Do not pre-cool the moulds 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 unpolymerised 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 catalyzed Infiltration Solution for the embedding solutions.

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

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

Mix 25ml of freshly made Infiltration Solution and 1.0ml of JB-4 Solution B thoroughly and begin embedding immediately. The small JB-4 Embedding Moulds from Polysciences, Inc. require approximately 1.5 to 2ml of solution per mould. The Block Holder is essential to exclude oxygen during the polymerization process. If Block Holders are not used, cover the moulds with an airtight film or place under vacuum at no more than 15psi, preferably in a cold room at 4°C or a refrigerator. If anaerobic conditions are not maintained, the JB-4 may polymerize incompletely or not all. BEEM® capsules may be capped for embedding. 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, therefore large blocks should be polymerized in the refrigerator or on ice. The blocks may range in colour from light yellow to dark yellow or amber. This colour shift is not a problem and will not affect 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.

Deplasticising 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.

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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 hands or exposed areas 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.

First Aid

In case of contact with any component or mixed solution immediately flush area with water for at least 15 minutes. Should either unpolymerised 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 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, plasticised benzyl 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 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.

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JB-4™ Embedding Kit

Background

Tissue embedded in JB-4TM Embedding Medium offers superior ultrastructural preservation and semi-thin sections for high resolution Light Microscopy. This water-soluble plastic media provides higher clarity and contrast than paraffin sections do, and offers the advantage of less distortion and tissue shrinkage for improved diagnostic capabilities.1, 2 Difficult specimens, such as undecalcified bone and delicate embryonic tissue can be easily processed in JB-4TM plastic.3

Both aqueous and non-aqueous fixatives can be utilized with JB-4TM embedding medium including Poly/LEM, Bouins, B-5 and neutral buffered fixatives. Unlike paraffin, removal of JB-4TM plastic prior to staining is unnecessary and brilliantly clear staining has been achieved using water-soluble dyes.4-9 JB-4TM does not interact with any tissue group of importance for staining during polymerization, in contrast to epoxy resins which may alter the staining character of tissue and are less responsive to histological stains.

Extremely fast processing times are possible with JB-4TM embedding medium as only a few simple processing steps are required. Since JB-4TM is water soluble, there is no need to completely dehydrate the tissue before infiltrating. A graded series of ethyl alcohols through 95% is commonly used to dehydrate tissue. Xylene, benzene or toluene are not required for dehydration. For the retention of lipids and enzymes, processing tissue at 4°C is recommended.10-11

On a routine basis, 1-2µm thick sections are easily cut with a glass knife. Recently, some investigators have even utilized conventional rotary microtomes for semi-thin sections of JB-4TM embedded material.12 Under the same conditions, paraffin sections must be cut 5-6µm thick with much cellular overlap and the loss of clear cellular resolution.

JB-4TM has been used successfully in enzyme histochemistry, immunohistochemistry, immunofluorescent techniques13-15 and high resolution autoradiography. Compared to conventional paraffin autoradiography sections, JB-4TM plastic enhances the ability to identify labeled cells.16

The increased nuclear differentiation obtained with JB-4TM plastic is a great aid to diagnosis, especially with liver and kidney tissue, where fine cytological detail and rapid processing is necessary.17 Such morphological evaluation from needle biopsies is not possible with paraffin or other plastic materials.18

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