

## Product Information

### JB-4 Embedding Kit®

Catalog Number **EM0100**  
Store at Room Temperature

## TECHNICAL BULLETIN

### Product Description

The 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 medium, JB-4 does not require dehydration to absolute alcohol except for dense, bloody, or fatty tissue specimens. It is excellent for small non-decalcified bone specimens, routine stains, special stains, and histochemical staining. Clearing agents such as xylene and chloroform are not required. Sections of JB-4 embedded material can be cut at 0.5–3.0 microns or thicker. Microtomes designed for plastic sectioning are required, as are glass, Ralph, or tungsten carbide knives.

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, the Osteo-Bed Bone Embedding Kit (Catalog Number EM0200) is recommended. The Osteo-Bed Bone Embedding Kit has a methyl methacrylate formulation, which is well suited for bone or soft tissue for immunohistochemistry.

### Components

JB-4 Solution A Monomer Catalog Number J4954	800 ml
JB-4 Solution B Accelerator Catalog Number J0330	30 ml
Benzoyl Peroxide, Plasticized Catalyst Catalog Number J0455	12 g

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

### Preparation Instructions

Preparation of Infiltration Solution – The following quantities are used to prepare 100 ml of Infiltration Solution:

JB-4 Solution A (Monomer)	100.0 ml
Benzoyl Peroxide, Plasticized (Catalyst)	1.25 g

Carefully weigh 1.25 g of the catalyst (benzoyl peroxide, plasticized) and add to 100.0 ml of JB-4 Solution A while stirring on a magnetic stirrer. Mix until dissolved, 10–20 minutes. Measurement of the catalyst is critical because it controls the rate of polymerization of the plastic and the exothermic reaction. This Infiltration Solution can be stored for up to two weeks in a cool, dark area or in the refrigerator at 2–8 °C.

Preparation of Embedding Solution – Prepare 25 ml of fresh Infiltration Solution.

Note: Do not use old or used catalyzed Infiltration Solution for the embedding solutions.

The following quantities are used to prepare 25 ml of Embedding Solution:

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

Just prior to the Embedding Procedure, mix 25.0 ml of freshly prepared Infiltration Solution and 1.0 ml of JB-4 Solution B thoroughly and begin embedding immediately.

### **Storage/Stability**

Store the kit in the original containers at room temperature in a cool, dark area. Refrigeration is not required. Do not store in the light or in a heated area as this may cause the monomer to polymerize.

The catalyst, plasticized benzoyl peroxide, is an organic peroxide that does not require special storage. The catalyst is formulated to remain active and be weighed correctly for this procedure without any adjustments to the amounts recommended. It should be kept tightly sealed. The catalyst may decompose with age; therefore, it is recommended to carefully monitor the date received and only use the catalyst with the kit in which it came for best results.

### **Procedure**

#### Fixation

Specimens can be fixed in 10% Neutral Buffered Formalin or other routine histological fixative. Routine specimen sizes for soft tissue should be no more than 2.0 cm × 2.0 cm × 2.0 cm 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 2–8 °C. Cold fixation will extend the time required for the specimen to be penetrated and fixed. Decalcification is not required for JB-4 embedded specimens.

#### Dehydration

Dehydration can be completed at room temperature or 2–8 °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 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.

**Note:** Dehydration and infiltration can be completed simultaneously by using absolute alcohol in ascending grades of Infiltration Solution starting at 50% ethanol and 50% Infiltration Solution, followed by 25% ethanol and 75% Infiltration Solution, then 10% ethanol and 90% Infiltration Solution, and finally in Infiltration Solution for a minimum of 3 changes prior to embedding. Proceed to the Embedding Procedure.

### Infiltration

Infiltration is performed at room temperature or 2–8 °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 8–10 times the volume of the specimen. The changes of fluid should occur every 10–90 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. Prior to preparing the Embedding Solution, collect and prepare the following materials:

- embedding molds
- block holders
- labels
- gloves
- instruments
- ice bath
- specimens

Do not precool the molds as this may cause condensation and prevent even polymerization of the block face. To prevent polymerization from occurring too rapidly and possible overheating of the tissue, it is recommended the polymerization process for embedding be slowed by completing it in a refrigerator, ice bath, or cold room at 2–8 °C. This may extend the polymerization from several hours to overnight.

Larger specimens using more volume (10–50 ml) of the Embedding Solution may have an even greater exothermic reaction, with the temperature of the reaction exceeding 100 °C at room temperature. Therefore, large blocks should be polymerized in a refrigerator, ice bath, or cold room at 2–8 °C. These larger specimens will require longer times for complete polymerization and may have more unpolymerized liquid on top of the block. This will appear as a syrup and should be allowed to drain off the blocks by inverting the molds upside down and then wiping off the excess. It is recommended that gloves be worn to reduce exposure to unpolymerized resin.

The block holder is essential to exclude oxygen during the polymerization process. If block holders are not used, cover the molds with an airtight film or place under vacuum at no more than 15 psi, preferably in a refrigerator, ice bath, or cold room at 2–8 °C to reduce the temperature of the exothermic reaction to  $\leq 55$  °C. If anaerobic conditions are not maintained, the JB-4 may polymerize incompletely or not at all. Room temperature polymerization will be complete in 1–2 hours for smaller blocks and may require three hours or more for very large blocks.

The blocks may range in color from light yellow to dark yellow or amber. This color 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.

#### Deplasticizing and Staining

JB-4 is a glycol methacrylate based polymer and 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.

JB-4 Embedding Kit is a registered trademark of Polysciences, Inc.

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