

# Immuno-Bed Kit

Catalog #17324

## Introduction

The Immuno-Bed embedding formulation is designed to allow easier penetration of lower molecular weight immunoglobulins and chromogens through the polymerized matrix for demonstration of antigenic sites. Tissue infiltration, embedding, and sectioning procedures are very similar to JB-4 Embedding Kits.

A semi-water-soluble media, Immuno-Bed does not require dehydration to absolute alcohol as it contains some glycol methacrylate. Immuno-Bed is excellent for routine stains, special stains, and histochemical staining. Clearing agents such as xylene and chloroform are not required or recommended. The polymerization of Immuno-Bed is exothermic, which is easily controlled by polymerizing on ice or by using refrigeration at 4°C. Immuno-Bed Embedding Kits must be used under a chemical fume hood. Immuno-Bed is compatible with most routine fixation procedures.

Immunostaining procedures that use lower molecular weight antibodies and chromogens are compatible with Immuno-Bed. For higher molecular weight antibodies and chromogens we recommend the Polysciences, Inc. Osteo-Bed Bone Embedding Kit (Cat. #17734). The Osteo-Bed formulation is a methyl methacrylate that is well suited for bone or for immunohistochemistry on routine histological specimens.

Immuno-Bed specimens can be sectioned at 0.5 to 3.0 microns or thicker and microtomes designed for plastic sectioning are required. Glass, Ralph, or tungsten carbide knives are recommended for sectioning. Tungsten carbide knives in several sizes and types are available from Polysciences, Inc.

**NOTE:** A tissue processor can be used and stopped at the end of the last alcohol step for dehydration steps. The tissue is removed to a fume hood for infiltration and embedding steps. Monomers should be used under a fume hood with appropriate gloves. Using monomers such as glycol and methyl methacrylate can void the manufacturer's warranty on tissue processors, see Warnings and Precautions. Check with the manufacturer prior to attempting infiltration on any unit.

## 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. Fixatives containing metals are not recommended.

Routine specimen sizes should be no more than 2.0cm X 2.0cm X 2.0cm with fixation at a minimum of four hours. Dense or fatty tissue may require overnight fixation after grossing to size. Fixation can be completed at room temperature or 4°C. Cold fixation will extend times slightly.

## Dehydration

Dehydration can be completed at room temperature or 4°C. This process can also be done with a routine processor programmed for small tissues and removed at the end of the last alcohol step.

## 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 time in each change is dependent on the size and density of the specimen and can range from as short as 10 minutes for smaller specimens to as long as 90 minutes for larger specimens. We strongly recommend that the user determine the optimal infiltration time for their situation. When infiltration is complete the tissue generally appears translucent and in most cases will sink to the bottom of the container. To allow complete saturation, infiltration should be done on a slow rotator, hematology shaker table, or inverted several times during the infiltration process.

Should any of our materials fail to perform to our specifications, we will be pleased to provide replacements or return the purchase price. We solicit your inquiries concerning all needs for life sciences work. The information given in this bulletin is to the best of our knowledge accurate, but no warranty is expressed or implied. It is the user's responsibility to determine the suitability for his own use of the products described herein, and since conditions of use are beyond our control, we disclaim all liability with respect to the use of any material supplied by us. Nothing contained herein shall be construed as a recommendation to use any product or to practice any process in violation of any law or any government regulation

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## Infiltration Solution Mixing Procedure

The following amounts of material are used for one 100ml of infiltration solution.

Immuno-Bed Solution A (Monomer)	100.00ml
Benzoyl Peroxide, Plasticized (Catalyst)	1.25gm

Carefully weigh 1.25gm of catalyst (benzoyl peroxide, plasticized). Mix until dissolved, approximately 10 to 15 minutes on a magnetic stirrer tightly capped. Measurement of the catalyst is critical, as it will control the rate of polymerization of the plastic and the exothermic reactions. Excess infiltration solution can be stored at 4°C for several weeks.

## Embedding Solution and Procedure

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

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 require greater amounts of embedding solution and this may result in an even higher temperature exothermic reaction. For this reason embedding of larger specimens are performed at 4°C. Also, larger specimens will require longer times for complete polymerization and may have more unpolymerized liquid on top of the block.

All embedding of specimens must be completed under anaerobic conditions only. (For 25 ml of embedding solution or multiply as needed for larger volumes)

Infiltration Solution 25.0ml

Carefully measure 25ml of Immuno-Bed Solution A (monomer)  
Weigh 0.32 grams of benzoyl peroxide, plasticized exactly

Stirred on a magnetic stirrer for 10 to 15 minutes or until completely dissolved.

Immuno-Bed Solution B (Accelerator) 0.25 ml [250µ] (Must be an exact measurement. Using a micro-pipetter is recommended for accuracy)

This solution must be used immediately as it will begin to polymerize within 10 to 20 minutes at room temperature.

The small 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 must be capped for embedding. The blocks should be clear and colorless to pale yellow. The top of the block may have a liquid film on it that can be removed by draining or drying the block in a desiccators for several hours to overnight.

## Deplasticizing and Staining

Immuno-Bed contains some glycol methacrylate that cannot be removed completely from the sections. Sections are picked up from a water bath on a clean slide and air-dried. Washing with 100% ethanol for 30 seconds to 2 minutes will soften and remove some of the polymerized matrix. The slides can be rehydrated quickly through a series of descending alcohols to water or buffer prior to staining. Routine histological stains can be performed on these slides or immunohistochemical procedures can be done following the same steps as paraffin embedded specimens. Staining times for Immuno-Bed samples may increase and should be carefully monitored to assure the reaction. Digestion with trypsin or protease K can be done after the first alcohol step to enhance the permeability of the sample for immunostaining.

## Warning

Immuno-Bed solutions may be harmful if swallowed. Use only under a hood and with appropriate gloves. Components may cause irritation or allergic skin reaction. Avoid contact with eyes, skin or clothing. Avoid inhalation of vapors. Wash thoroughly hands or exposed areas at once, after handling.

## Precautions

Do not heat over an open flame. Avoid electrical or static sparks. Polymerize only in an electric oven meeting all codes for explosion proof operation. Store un-catalyzed resins at room temperature in the original containers.

## Storage

Refrigeration of the kit components is not required. The components 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 the catalyst in 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.

### First Aid

In case of contact, with any component or mixed solution immediately flush area with water for at least 15 minutes. Should either un-polymerized 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.

### Ordering Information

Catalog #	Description	Size
17324-1	Immuno-Bed Embedding Kit	1 Kit
17325A-800	Immuno-Bed Solution A (Monomer)	800ml
17325B-30	Immuno-Bed Solution B (Accelerator)	30ml
17325C-12	Immuno-Bed Benzoyl Peroxide, Plasticized (Catalyst)	12.0g
16864-3.75	Poly/LEM, Methanol Free Fixative	3.75L
16864-4	Poly/LEM, Methanol Free Fixative	4 x 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-3	Polyethylene Molding Cup Trays 6 X 8 X 5mm (9 Cavities) Hex	3 Each

Catalog #	Description	Size
17177B-3	Polyethylene Molding Cup Trays 2 X 15 X 5mm (9 Cavities)	3 Each
17177C-3	Polyethylene Molding Cup Trays 13 X 19 X 5mm (9 Cavities)	3 Each
15899-50	JB-4® Plastic Block Holders	50/pkg
24216-1	Tissue Tack Slides Silane Coated (Approx. 72 slides per box)	1 box
24234-1	Tungsten Carbide Disposable Blades	2/pkg
24235-1	Tungsten Carbide Disposable Blades (2) and 1 Reusable Blade Holder	1 pkg
24233-1	Triangular Tungsten Carbide Knives	1 pkg

### To Order

In The U.S. Call: 1-800-523-2575 • 215-343-6484  
 In The U.S. FAX: 1-800-343-3291 • 215-343-0214

In Germany Call: (49) 6221-765767  
 In Germany FAX: (49) 6221-764620

Order online anytime at [www.polysciences.com](http://www.polysciences.com)

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