

Osteo-Bed Plus Embedding Kit

Catalog #24889

Introduction

Osteo-Bed Plus Embedding Kit is a formulation for embedding large and small undecalcified bone specimens. Undecalcified bone sections provide the investigator with reliable material for the diagnosis and investigation of metabolic bone diseases, particularly osteomalacias. The Osteo-Bed Plus embedding formulation is also recommended for immunohistochemistry procedures performed on trephines and other non-decalcified bone or soft tissues. Osteo-Bed Plus is a methyl methacrylate-based material that can be removed from the section and allows the use of staining procedures very much like a paraffin section. Large bone samples embedded with Osteo-Bed Plus should be sectioned with a heavy-duty microtome. Small bone and soft tissue embedded with Osteo-Bed Plus should be sectioned with a microtome designed to cut plastic embedded materials.

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

Fixation

Bone specimens can be fixed in alcoholic formalin or 10% neutral buffered formalin (NBF, Cat. #08379). Poly/LEM (Cat. #16864) is methanol free 10% neutral buffered formalin based fixative for light and electron microscopy. The usual fixation time is 48 hours or as needed for large samples such as femoral heads. Smaller specimens (<1.0 cm³) may be fixed in less time. Times should be determined by individual specimen sizes and types. The blocks should be grossed prior to determining the fixation time is complete. Larger specimens can require additional fixation time after trimming to the correct size for processing.

Fixative, dehydrants, and infiltration solutions should be used at 8 to 10 times the volume of fluid to specimen in each change.

Dehydration:

Large samples can be dehydrated at 4°C or room temperature. Using a tissue processor will allow less "hands on" time and standardization of the process for the tissues. It is not a requirement to use a cold dehydration for these steps for either small or large specimens.

If using a tissue processor, the program can be adjusted to stop at the last clearing step and allow no paraffin infiltration.

The following is a tissue processor example and can be used for manual processing with some agitation during the steps added. Tissue will be pre-fixed and can start from the first alcohol step.

70% Ethanol	1 change	1 to 2 hours
<i>(or as a hold for delayed starts)</i>		
80% Ethanol	1 change	1 to 2 hours
95% Ethanol	2 changes	1 to 4+ hrs for each change
100% Ethanol	2 changes	1 to 4+ hrs for each change
Xylene	1 change to defat the specimen	at 1 to 2 hours only
Xylene substitute (Clear-Advantage Cat. #24770 or Isopar Type)	2 changes	at 2 to 4 hours each

Remove from the tissue processor and begin infiltration steps.

Xylene should be used to defat the specimen. Fat will become dense spots in the tissue causing knife damage if not removed from the bone. Xylene substitute (Clear-Advantage Cat. #24770 or Isopar Type) should be used to remove the xylene.

(Isopar derivatives will give a slightly pliable feel to the bone. The ability to have bone become less friable allows better sectioning. The d-limonene substitutes are not as effective in this or any plastics procedure. Fat will become dense spots in the tissue causing knife issues if not removed from the bone.)

Small samples (*small tissues, bones or trephines*) are dehydrated at room temperature on a tissue processor or manually.

70% Ethanol	1 change	30 minutes to 1 hour
80% Ethanol	1 change	20 minutes to 1 hour
95% Ethanol	2 changes	30 minutes to 1 hour each
100% Ethanol	2 changes	30 minutes to 1 hour each

Xylene at one change to defat the specimen 20 minutes to 30 minutes. Xylene substitute (Clear-Advantage Cat. #24770 or Isopar Type) for 2 changes at 1 hour to 2 hours each to remove the xylene and clear the specimens.

Infiltration

Infiltration with Osteo-Bed Plus Embedding Media is completed in three parts over several days for large specimens. Small specimens may be infiltrated in one day or overnight. Specimens can be completed at room temperature or at 4°C. Room temperature infiltration steps should be completed under a hood with as little exposure to direct light as possible.

Step 1: Use Osteo-Bed Plus Resin Only

Use a minimum of 2 changes over 8 to 12 hours (overnight is acceptable for ease of timing factors for small specimens and 24 to 48 hours for large specimens.) Timing should be based on specimen size and laboratory procedures.

Use appropriate gloves when handling Osteo-Bed Plus resin as it can cause skin irritation. All mixing and handling of any resin should be performed under a hood.

Step 2: Preparing Pre-polymerized Layers for Ease in Trimming and Sectioning

These should be prepared prior or during the processing and infiltration steps. The layers can be made and stored for future use in various sizes of containers. Pre-polymerized layers are used to avoid the specimen sitting directly of the bottom of the "mold" or container. It allows better orientation of specimens sectioning.

Note: Avoiding glass containers is a safety measure to avoid breaking the container away from the completed polymerized block. Plastic containers can be used to replace the glass often used and are removed by cutting or using a saw to remove them from the block. Any container selected must have a tight fitting lid to eliminate air during polymerization in all steps.

Prepare the amount of embedding solution required for pre-polymerized layers. (*See step 2 for directions.*) These can be prepared in flat bottom high density polyethylene (HDPE) containers with tight fitting caps in the size required for either large or small specimens. This step prevents the specimen laying directly on the bottom of the container for improved sectioning. The layers should be sufficient to allow at least one quarter to one half inch from the bottom of the container for support. The block is cut from the HDPE container with either a safety knife or preferably with a saw. The block is trimmed for sectioning usually with a saw or by the protocol used in the laboratory.

Note: The layers and all blocks can be polymerized in a water bath filled to the appropriate level with sand. This avoids the containers floating and/or turning over in water. The temperature should be between 32°C to 34°C for either. Using a sand bath instead of a water bath allows little or no condensation to appear in the containers during polymerization of the layers or blocks.

Step 3: Infiltration with Osteo-Bed Plus Solution A Resin Only

Infiltration Procedure for Small or Large Specimens:

Small Specimens – 2 changes over 3 to 12 hours of Osteo-Bed Plus Solution A Resin only

Large Specimens – 2 to 3 changes in 24 to 36 hours of Osteo-Bed Plus Solution A Resin only

Step 4: Catalyzed Osteo-Bed Plus Resin Used for Infiltration Only. (*Catalyzed Solutions have benzoyl peroxide added*)

Catalyzed Osteo-Bed Plus Infiltration Solution

Osteo-Bed Plus Solution A Resin: 100ml
Benzoyl peroxide, plasticized 1.40gm

Add 1.40gm of benzoyl peroxide to 100ml of Osteo-Bed Plus Resin in a container with a tight cap on a magnetic stirrer.

(For large volumes of catalyzed infiltration solution pour 900ml of Osteo-Bed Plus Resin into a one liter HDPE bottle and add 12.60gm or one bottle of Benzoyl Peroxide Plasticized (Cat. #24232) to the bottle.)

Stir for a minimum of 4 hours.

This solution can be stored at 4°C for several weeks. Tightly cap all stored resin to protect from moisture. Do not store excess catalyzed resin at room temperature. Infiltration steps are completed at room temperature in the dark or minimum light exposure. Stir for a minimum of 6 to 8 hours for best results. Keep specimen in catalyzed infiltration solution for minimum of 6 to 8 hours.

Note: Using the glass bottle can cause issues if the solution polymerizes and breaks the bottle due to heat or nature. Place the bottle in a 1000ml HDPE container to avoid any leaks. Glass is not recommend for any step due to the dangers of breakage while liquid or polymerizing.)

Step 5: Embedding Solution and Embedding Steps

(*See Step 2 for preparing Pre-polymerized layers*)

Catalyzed Osteo-Bed Plus Resin for large and small specimens

100ml of Osteo-Bed Plus Resin
Add 3.5gm of Benzoyl peroxide, plasticized

Stir for a minimum of 6 to 8 hours for best results. Increase volumes as needed for larger amounts of embedding solution. This solution can be stored at 4°C overnight or for several weeks. Tightly cap all stored resins to protect from light and moisture. (The solution should be at room temperature to begin this procedure.)

Do not store excess catalyzed resin at room temperature.

Embedding Procedure: (Using pre-polymerized layers from step 2 prior to beginning the embedding procedure.)

Note: Polymerization is exothermic and the temperature can rise very quickly during polymerization in surrounding bath of water or sand. Separate containers by a minimum of 1.5 inches during the polymerization step.

Place the infiltrated specimen on the pre-polymerized resin layer and fill the container to a level that will cover the specimen and a top for the block for mounting on a microtome chuck. Do not fill the container completely as it may break during polymerization.

Allow to sit overnight at room temperature under a hood prior to placing in the bath or oven for polymerization. This allows time for a secondary infiltration of embedding solution.

Note: Maintaining the temperature is critical for complete polymerization with reduced exothermic reaction, which may cause bubbles or damage the specimens.

Temperature must be maintained at 32°C to 34°C +/- 0.5°C whether polymerizing in a water bath or prepared water bath with sand. Ovens generally are not well controlled to maintain this low temperature and can over heat easily. A sand bath can be used in the oven to assist in maintaining the temperature. Water should not be used in an oven as it will create condensation in the container and oven during polymerization and cause electrical problems due to water build up.

The specimen container should be covered to a level above that of the Osteo-Bed Plus Embedding Solution during this step. Sand allows the container to be covered with no weights to maintain the container upright during polymerization. Water will require weights to assure the upright position is maintained.

Do not move or disturb the molds or vessels containing the specimen and embedding solution during polymerization. Osteo-Bed Plus will polymerize by forming layers from the bottom to the top. Disruption of the layers may cause the material to polymerize poorly and not form a solid block. Total polymerization time may be 12-48 hours or occasionally longer with larger volumes.

A small amount of un-polymerized resin may be present on top of the block. This may be soft or slightly liquid and can be removed by wiping or scraping the layer off. If the level of embedding solution falls below the specimen an additional amount of embedding solution can be added and the container returned to the sand or water bath to allow better coverage.

Occasionally the exothermic reaction will become active too quickly and bubbles may form causing the block to appear like cracked

ice. The excess material can be removed by sawing or trimming as close to the sample as possible. Repeat steps 1 and 3 through 5 to correct the problem. Step one is used to remove any excess polymerized plastic around the specimen. Re-embed as directed.

If a glass jar or container has been used, place it in a refrigerator to cool completely. Wrap the jar in a towel to protect hands and eyes and gently tap with a hammer to break the polymerized specimen free. Trim the block with a saw to fit the microtome Block holder. Smaller blocks can be removed from the molds and sectioned.

Deplasticizing and Staining

Most histochemical and immunohistochemistry staining protocols require the removal of methyl methacrylate (MMA) and rehydration of mounted sections prior to staining in order to provide deeper penetration and greater contrast.

Work under a hood or in a very well ventilated area.

Variation 1:

1. Osteo-Bed Plus Solvent at 37-45 degrees Celcius.
2. Three changes of Osteo-Bed Plus solution with 15-30 minutes between changes.
3. The last change should be acetone before hydrating the slides
4. Procedure may also be done at room temperature and sections may be allowed to soak overnight.

Variation 2:

50% acetone and 50%Osteo-Bed Plus Solvent

1. Change solutions three times with 15-30 minutes between changes.
2. The last change should be left overnight in acetone

Procedure is done at 45° C.

Rehydrate your sections by using the following series:

- 100% alcohol, two times, 15 minutes each.
- 95% alcohol, two times, 5 minutes each.
- 80% alcohol, three times, 5 minutes each.
- 70% alcohol, three times, 5 minutes each.
- 100% distilled water.

Sections are now ready for staining or immunolabeling.

Warning

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 hands thoroughly 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 uncatalyzed resin at room temperature in the original container.

First Aid

In case of contact, immediately flush eyes with water for at least 15 minutes. Flush skin or exposed areas with water for 15 minutes. If swallowed, dilute by drinking water to excess. Call a physician immediately. Never give anything by mouth to someone who is unconscious.

Disposal of Benzoyl Peroxide Catalyst

The catalyst may be destroyed by adding it in small portions to cold 10% sodium hydroxide solution. Use four times the volume/ weight of liquid to catalyst. Do not allow the material to settle or form clumps. Dispose of this solution and Osteo-Bed Plus Resin and solutions along with hazardous wastes in accordance with local, state, and/or federal regulations.

Ordering Information

Catalog #	Description	Size
248891	Osteo-Bed Plus Embedding Kit	1 kit

Additional Products for Use with Osteo-Bed Plus Kit:

Catalog #	Description	Size
16864-1	Poly/LEM, Methanol Free Formalin Fixative	3.75 L
16864-3.75	Poly/LEM, Methanol Free Formalin Fixative	4 x 3.75 L
24216-1	Tissue Tack Microscope Slides (approx. 72)	1 box
24234-1	Tungsten Carbide Disposable Blades	2 per pkg.
24235-1	Tungsten Carbide Disposable Blades(2) and 1 Reusable Blade Holder	1 kit
24233-1	Triangular tungstun carbide knives	3 per pkg.
08379-3.75	Formalin, 10% neutral, phosphate buffer (NBF)	3.75 L
08379-20	Formalin, 10% neutral, phosphate buffer (NBF)	20 L
09860-1	Alcohol Reagent, histology grade	1 gal
08389-1	Xylene, histology grade	1 gal
22247-1	Poly-L-Lysine Coated Microscope Slides	1box

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