

Product Information

Osteo-Bed Bone Embedding Kit

Catalog Number **EM0200**
Store at Room Temperature

TECHNICAL BULLETIN

Product Description

The Osteo-Bed Bone 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 embedding formulation is also recommended for immunohistochemistry procedures performed on trephines and other non-decalcified bone or soft tissues.

The Osteo-Bed formulation is methyl methacrylate-based and can be removed from the section, allowing the use of staining procedures similar to a paraffin section. Large bone samples embedded with Osteo-Bed should be sectioned with a heavy-duty microtome. Small bone and soft tissue embedded with Osteo-Bed should be sectioned with a microtome designed to cut plastic embedded materials.

Components

Osteo-Bed Resin, Solution A Catalog Number O8514	900 ml
Osteo-Bed Bone Embedding Catalyst Catalog Number O8764	2 × 12 g

Reagents Required but Not Provided

- Osteo-Bed Bone Embedding Solvent (Catalog Number O8639)
- Acetone

Precautions and Disclaimer

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

Preparation Instructions

Any container used must have a tight-fitting lid to eliminate air. Glass is not recommended due to the danger of breakage.

Catalyzed Osteo-Bed Infiltration Mixture – Prepare 100 ml by adding 1.4 g of Osteo-Bed Bone Embedding Catalyst (Catalog Number O8764) to 100 ml of Osteo-Bed Resin (Catalog Number O8514) in a container with a tight-fitting cap and a magnetic stir bar. Stir for a minimum of 4 hours on a magnetic stirrer. The prepared mixture will be cloudy.

Catalyzed Osteo-Bed Embedding Mixture – Prepare 100 ml by adding 3.5 g of Osteo-Bed Bone Embedding Catalyst (Catalog Number O8764) to 100 ml of Osteo-Bed Resin (Catalog Number O8514) in a container with a tight-fitting cap and a magnetic stir bar. Stir for a minimum of 6 hours on a magnetic stirrer. The prepared mixture will be cloudy.

Store both prepared mixtures at 2–8 °C immediately after preparation. Prepared mixtures may be stored at 2–8 °C for up to 4 weeks. Tightly cap all stored resin to protect from moisture. Do not store excess catalyzed resin mixtures at room temperature.

Storage/Stability

Store the kit at room temperature.

Procedure

Specimens can be infiltrated at room temperature or at 2–8 °C. The volume of infiltration solutions used should be ~10 times the specimen volume. Infiltration steps should be completed under a fume hood with as little exposure to direct light as possible.

1. Bone samples should be fixed and dehydrated following established processing schedules determined by individual specimen sizes and types. Xylene may be used to defat the specimens. 100% Ethanol should be used to remove excess xylene.

2. Using Osteo-Bed Resin, Solution A (Catalog Number O8514) **only**, infiltrate small specimens with a minimum of 2 changes over 3–12 hours and large specimens with a minimum of 2 changes over 36 hours. Exact timing should be based on specimen size and laboratory procedures.
3. After completion of initial infiltration (step 2), infiltrate specimen for a minimum of 6 hours using the prepared Catalyzed Osteo-Bed Infiltration Mixture.
4. To allow better orientation of specimens for sectioning, molds with a pre-polymerized resin layer may be prepared. Add a layer of Catalyzed Osteo-Bed Embedding Mixture to the embedding mold and heat using a water bath at 50 °C for up to 2 hours. This will produce a viscous layer capable of supporting the specimen.
5. Place the infiltrated specimen in a container with or without a pre-polymerized resin layer.
6. Embed the specimen with room temperature Catalyzed Osteo-Bed Embedding Mixture to a level that will cover the specimen.
7. Allow specimen to sit overnight at room temperature under a fume hood prior to raising the temperature for polymerization. This allows time for a secondary infiltration of the embedding solution.
8. Polymerize the Catalyzed Osteo-Bed Embedding Mixture for a minimum of 24 hours by maintaining the specimen container temperature at 50–60 °C. Larger volumes may require a longer polymerization time. Do not disturb the container during polymerization.
Note: It may be necessary to degas the Catalyzed Osteo-Bed Embedding Mixture or to use a lower polymerization temperature over a longer time period to prevent bubble formation. Parameters of the procedure must be optimized by the user based on the nature of the specimen.
9. A small amount of unpolymerized resin may remain on top of the block. This can be removed by scraping the layer off. If the level of embedding mixture falls below the specimen, an additional amount of embedding mixture can be added.

Deplasticizing

Many histochemical and immunohistochemistry staining protocols require the removal of methyl methacrylate (MMA). Osteo-Bed Bone Embedding Solvent (Catalog Number O8639, not provided with this kit) may be used to remove the polymerized resin.

1. Deplasticize the embedded sections using Osteo-Bed Bone Embedding Solvent at 27–37 °C and acetone.
 - a. Three changes of Osteo-Bed Bone Embedding Solvent with 15 minutes between changes. Larger specimens may require additional changes of solvent for longer time periods.
 - b. The final change should be acetone before hydrating the sections.
2. Rehydrate sections using established processing schedules determined by individual specimen sizes and types

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