**Product Information**

**Osteo-Bed Bone Embedding Kit**

Catalog Number **EM0200**

Store at Room Temperature

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**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 900 ml
  Catalog Number O8514

- Osteo-Bed Bone Embedding Catalyst 2 \( \times \) 12 g
  Catalog Number O8764

**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**

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 Solution – 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 cap and a magnetic stir bar. Stir for a minimum of 6 hours on a magnetic stirrer.

These solutions can be stored at 2–8 °C for several weeks. Tightly cap all stored resin to protect from moisture. Do not store excess catalyzed resin at room temperature.

**Procedure**

To allow better orientation of specimens for sectioning, molds with a pre-polymerized layer using Catalyzed Osteo-Bed Embedding Solution should be prepared prior to the infiltration steps.

Specimens can be infiltrated at room temperature or at 2–8 °C. The volume of infiltration solutions used should be 8–10 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 a minimum of 2 changes over 24–36 hours. Exact timing should be based on specimen size and laboratory procedures.

3. Using the prepared Catalyzed Osteo-Bed Infiltration Solution, infiltrate specimen for a minimum of 6 hours.
4. Place the infiltrated specimen in a container with or without a pre-polymerized resin layer.
5. Embed the specimen with room temperature Catalyzed Osteo-Bed Embedding Solution to a level that will cover the specimen.
6. 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.
7. Polymerize the Catalyzed Osteo-Bed Embedding Solution for 12–48 hours by maintaining the specimen container temperature in the range of 31.5–34.5 °C. Larger volumes may require a longer polymerization time. Do not disturb the container during polymerization.
8. 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 solution falls below the specimen, an additional amount of embedding solution can be added.

Deplasticizing and Staining

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.

Option 1 – Deplasticizing using Osteo-Bed Bone Embedding Solvent at 37–45 °C. 

Note: This option may also be done at room temperature and sections may be allowed to soak overnight.

1. Three changes of Osteo-Bed Bone Embedding Solvent with 15–30 minutes between changes.
2. The final change should be acetone before hydrating the slides.

Option 2 – Deplasticizing using a mixture of 50% acetone and 50% Osteo-Bed Bone Embedding Solvent at 45 °C.

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

Rehydrate sections using established processing schedules determined by individual specimen sizes and types.

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