

Product Information

Villanueva Osteochrome Bone Stain

Catalog Number **SHH0027**

Store at Room Temperature

Product Description

Villanueva Osteochrome Bone Stain gives uniform and reproducible results for mineralized or undecalcified bone. It can be used in staining fresh or fixed, unembedded or plastic-embedded sections of bone.

This staining method has been used effectively in research of new bone formation in hydroxyapatite implants and bone grafts.

Villanueva Bone Stain does not require special equipment or complicated procedures. Bone specimens are fixed in 70% ethanol, but 10% buffered formalin may be used as alternative fixative. Avoid use of fixatives containing dichromates, heavy metals, or acid, which can interfere with the staining.

Additional Product Recommended but Not Provided

- Osteo-Bed Bone Embedding Kit (Catalog Number EM0200)

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.

Storage/Stability

Store Villanueva Osteochrome Bone Stain at room temperature. The solution is stable at room temperature for up to one year.

Procedures

A. Unembedded Samples

1. Cut bone into slabs of 2–3 mm thick.
2. Grind sections to 50–100 μm thick under gently running water.
3. Rinse sections in distilled water.
4. Stain in Villanueva Bone Stain for 48 hours for complete permeation of bone and tissue elements. Sections may be stained in Villanueva Bone Stain for 90 minutes for fast stains but with incomplete permeation of the section by the stain.
5. Transfer sections into tap water and then grind surface stains.
6. Wash sections with 0.01% mild household detergent.
7. Wash sections with tap water and then rinse with distilled water.
8. Differentiate in 0.01% glacial acetic acid in 95% methanol.
 - a. Treat for 3–5 minutes for samples stained for 90 minutes in Step 4.
 - b. Treat for 20–25 minutes for samples stained for 48 hours in Step 4.
9. Dehydrate in the following:
 - a. 95% alcohol for 15 minutes.
 - b. 100% alcohol for 15 minutes.
10. Clear in the following:
 - a. Equal parts of 100% alcohol and xylene for 10 minutes.
 - b. One part of 100% alcohol and 3 parts of xylene for 5 minutes.
 - c. One part of 100% alcohol and 9 parts of xylene for 5 minutes.
 - d. Xylene for 10 minutes.
 - e. Xylene for 5 minutes.
 - f. Xylene for 5 minutes.
11. Mount on slides using appropriate mounting medium.

B. Plastic-Embedded Sections

Note: To post-stain plastic embedded samples deplasticize slides for 4 hours in 55 °C xylene, then transfer through graded ethanols (100% to 70%), and then a final distilled water rinse before staining. Staining times may vary, being dependent on thickness of the section.

1. Cut fresh or fixed bone into slabs of 2–3 mm thick.
2. Stain in the Villanueva Osteochrome Bone Stain for 72 hours.
3. Dehydrate in the following:
 - a. 70% ethanol for one hour.
 - b. Two changes of 95% ethanol for 90 minutes each.
 - c. Two changes of 100% ethanol for 90 minutes each.
 - d. Acetone for 90 minutes.
4. The bone is then processed in methyl methacrylate (MMA). Alternatively, the Osteo-Bed Bone Embedding Kit may be used. The kit has been specifically formulated for embedding mineralized (undecalcified) bone specimens.

Results

Osteoid seams.....	Transparent green to jade green or homogeneous red
Zone of demarcation.....	Orange-red
Low-density bone	Red
Moderately permeable bone.....	Orange
Osteocytes, canaliculi, halo volumes	Red
Nuclei of osteoblasts, osteoblasts	Greenish-blue to dark purple
Cytoplasm.....	Green or light green

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