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TECHNICAL DATA SHEET 273

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Villanueva Osteochrome Bone Stain

USAGE:

Villanueva 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. In addition, it should prove useful in the study of age-related bone loss, osteoporosis and different metabolic bone diseases.

MATERIALS:

Villanueva Bone Stain is inexpensive to use since it does not require special apparatus or complicated procedures. Stock solutions are stable at room temperature for up to one year. Bone specimens are fixed in 70% ethanol, but 10% buffered formalin is permissible. Avoid fixatives containing dichromates, heavy metal or acid, which can interfere with the staining.

PROCEDURE: UNEMBEDDED

1. Cut bone into slabs of 2 to 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. 90 minutes for 1 or 2 sections and 49 hours for 2 to 4 sections.

Note: The 90 minutes staining yields fast stains of diagnostic usefulness but with incomplete permeation of the section by the stain; the 48 hour staining is for complete permeation of bone and tissue elements.

5. Transfer sections into tap water and then grind surface stains.
6. Wash sections with 0.01% mild household detergent. (Eg. Ivory Soap)
7. Wash sections with tap water and then rinse with distilled water.
8. Differentiate in 0.01% glacial acetic acid in 95% methanol (3 - 5 minutes for the 90 minute staining; 10 minutes for the 24 hours staining; 20 - 25 minutes for the 48 hour staining.)
9. Dehydrate in the following:
 - a. 95% alcohol for 15 minutes.
 - b. 100% alcohol for 15 minutes.

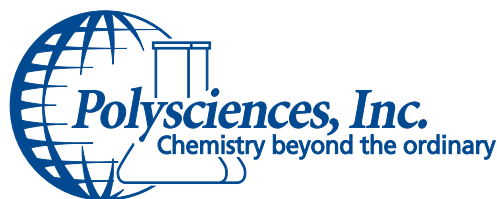
10. Clear in the following:

- a. Equal parts of alcohol, 100% plus 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 in Polymount (#08381) mounting medium.

PROCEDURE: (PLASTIC-EMBEDDED SECTIONS)

1. Cut fresh or fixed bone into slabs of 2 to 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 or 90 minutes.
4. The bone is then processed in methyl methacrylate (MMA). Alternatively, use Polysciences Osteo-Bed Bone Embedding Kit specifically formulated for embedding mineralized (undecalcified) bone specimens.



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RESULTS:

- Osteoid seamstransparent green to jade green or homogeneous red.
- Zone of demarcationorange-red
- Low-density bone.red
- Moderately permeable boneorange
- Osteocytes, canaliculi, halo volumesred
- Nuclei of osteoblasts, osteoblastsgreenish blue to dark purple.
- Cytoplasmgreen or light green.

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.

ORDERING INFORMATION:

Cat. #	Description	Size
16280	Villanueva Osteochrome Bone Stain	450mL
08381	Poly-Mount	120mL, 940mL
08032	Methanol (Methyl alcohol)- 99.5% EM Grade	6 x 1pt.
17734	Osteo-Bed Bone Embedding Kit - Data sheet #355	1 Kit
03573	Methyl Methacrylate - Butyl Methacrylate Embedding Kit - Data Sheet #408	1 Kit

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