

## Decalcifying Solution, Formic Acid/Formalin - Technical Memo

**SOLUTION:** Part 10493B Part 10493C  
Decalcifying Solution, Formic Acid/Formalin 1 liter 1 Gallon

**Additionally Needed:**  
Decalcification End Point Set Part 1051

**For storage requirements and expiration date refer to individual bottle labels.**

### APPLICATION:

Newcomer Supply Decalcifying Solution, Formic Acid/Formalin combines bone decalcification and fixation into a one-step time saving process. This solution provides good cellular morphology preservation with a moderate rate of decalcification that is designed for light bone specimens such as sinus contents and disc material. It is not recommended for femoral head and long bone sections.

### METHOD:

**Fixation:** Separate fixation is not required

**Technique:** Paraffin sections cut at 5 microns on adhesive slides

**Solutions:** All solutions are manufactured by Newcomer Supply, Inc.

### PROCEDURE:

- Submerge bone segment(s) in container of Decalcifying Solution, Formic Acid/Formalin that adequately covers the specimen. A 20:1 ratio is recommended.
  - See Procedure Notes #1 and #2.
- Check the specimen regularly for adequate solution coverage at routine intervals during the fixation/decalcification process for optimal reaction. Decalcification time will vary and is dependent on size and weight of bone.
  - Check light bone samples every 1 to 2 hours.
  - Light bone specimens, on the average, will fix and decalcify in 4 to 6 hours.
- Check completion of decalcification with Decalcification End Point Set (1051) regularly to deter over-decalcification and loss of cellular morphology.
  - See Procedure Note #3.
- Wash the specimen in running tap water when decalcification is judged to be complete. Suggested time for small samples is 30-60 minutes; larger bones 1-4 hours or according to laboratory protocol established times.
  - Additional trimming of decalcified bone can occur at this stage to a size and thickness suitable for tissue processing.
- Proceed with laboratory tissue processing procedure for bone specimens.
- Trim block(s) and section the processed, paraffin embedded bone; if block trimming or sectioning is impaired due to bone hardness, surface decalcification is recommended.
  - See Procedure Note #4.
- Perform surface decalcification by soaking the paraffin block with exposed tissue surface side down in recommended decalcifying solution for 15-60 minutes. Rinse block thoroughly with distilled water to remove corrosive acids and re-section.
  - See Procedure Note #5.

### PROCEDURE NOTES:

- Decalcification/fixative solution should be in contact with all specimen surfaces. If multiple pieces are in one container, ensure that pieces are separated and/or suspended and not in direct contact or stacked on top of each other. Change the solution at least daily and never add to or mix fresh solution with old.
- Fixation/decalcification can be enhanced with the use of low speed agitation with either a stir bar/stir plate or rotator/shaker.
- Decalcification end-point testing can also be accomplished through specimen radiography. Physical testing (probing or bending) of the bone is not recommended.
- Decalcifying Solution, Formic Acid/Formalin is not a preferred product for surface decalcification. Decalcifying Solution, Formic Acid 5%, Aqueous (1049) and Decalcifying Solution, Formic/Citrate (10492) are the recommended products for optimal surface decalcification.
- Surface decalcification removes only a thin layer of residual calcium from the tissue block surface. This will allow only a few calcium-free sections to be obtained. Repeating the surface decalcification process for additional sections may be required.

### REFERENCES:

- Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 338-343.
- Luna, Lee G. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. 3rd ed. New York: Blakiston Division, McGraw-Hill, 1968. 6-11.
- Urban, Ken. "Routine Decalcification of Bone." *Laboratory Medicine* 12.4 (1981): 207-212.
- Villanueva, Anthony. "Experimental Studies in Demineralization and Its Effects on Cytology and Staining of Bone Marrow Cells." *The Journal of Histotechnology* 9.3 (1986): 155-161.
- Modifications developed by Newcomer Supply Laboratory.