

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

**SOLUTION:**  
Decalcifying Solution, Formic Acid/Formalin

**1 Liter**  
Part 10493B

**1 Gallon**  
Part 10493C

**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 not required

**Technique:** Paraffin sections cut at 4 microns on adhesive slides

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

### PROCEDURE:

1. Submerge bone segment in Decalcifying Solution, Formic Acid/Formalin, adequately covering specimen at a 20:1 ratio.
  - a. See Procedure Notes #1 and #2.
2. Check the specimen regularly for adequate solution coverage. Change solution daily and do not add or mix fresh solution with old.
3. Decalcification time will vary, dependent on bone size and weight.
  - a. Check light bone samples every 1 to 2 hours.
  - b. Light bone specimens, on average, will fix and decalcify in 4 to 6 hours.
4. Check decal completion at regular intervals with Decalcification End Point Set (1051) to deter over-decalcification.
  - a. See Procedure Note #3.
5. Wash in running tap water when decalcification is complete.
  - a. Wash small samples 30-60 minutes.
  - b. Wash larger bones 1-4 hours.
  - c. Additional trimming of decaled bone can occur at this point to size and thickness suitable for tissue processing.
6. Proceed with tissue processing procedure for bone specimens.
7. Trim block and section bone. If trimming or sectioning is impaired due to bone hardness, surface decalcification is recommended.
  - a. See Procedure Note #4.
8. Perform surface decalcification: Soak exposed tissue surface side down in recommended decalcifying solution for 15-60 minutes. Rinse block with distilled water to remove corrosive acids and re-section.
  - a. See Procedure Note #5.

### PROCEDURE NOTES:

1. Decal solution should be in contact with all specimen surfaces. For multiple pieces, ensure pieces are separated or suspended and not in direct contact or stacked on each other.
2. Enhance fixation/decalcification with low-speed agitation shaker, rotator or stir plate.
3. Decalcification end-point testing can also be done with specimen radiography. Physical probing of bone is not recommended.
4. 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 recommended for optimal surface decalcification.
5. Only a few calcium-free sections will be obtained after surface decalcification. Repeat the process for additional sections.

### REFERENCES:

1. Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 338-343.
2. 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.
3. Urban, Ken. "Routine Decalcification of Bone." *Laboratory Medicine* 12.4 (1981): 207-212.
4. 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.
5. Modifications developed by Newcomer Supply Laboratory.