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Part 1049
Revised March 2025

Decalcifying Solution, Formic Acid 5%, Aqueous - Technical Memo

SOLUTION:	**1 Liter**	**1 Gallon**	**20 Liter Cube**
Decalcifying Solution, Formic Acid 5%, Aqueous	Part 1049B	Part 1049C	Part 1049E

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 5%, Aqueous, provides a moderate rate of decalcification while maintaining cellular morphology. This solution is a general purpose decalcifier and suitable for all bone specimen types from sternal or iliac crest bone marrow biopsies (light bone) to femoral head and long bone sections (compact bone).

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
- *a. See Procedure Note #1.*

Technique: Paraffin sections cut at 4 microns on adhesive slides
Solutions: All solutions are manufactured by Newcomer Supply, Inc.

PROCEDURE:

1. Fix bone for a length of time sufficient for specimen size and type.
   - *a. See Procedure Note #2.*
2. Adequate bone fixation is essential before decal solution exposure.
3. Wash fixed specimen in running tap water for 10 minutes.
4. Submerge fixed bone segment in Decalcifying Solution, Formic Acid 5%, Aqueous, covering specimen at a 20:1 ratio.
   - *a. See Procedure Notes #3 and #4.*
5. Check specimen regularly for sufficient solution coverage. Change solution daily and do not add or mix fresh solution with old.
6. Decalcification time will vary, dependent on bone size and weight.
   - *a. Check light bone samples every 30 to 60 minutes.*
   - *b. Check compact bone samples every 1 to 2 hours.*
   - *c. Bone marrow or light bone biopsies, on average, will decalcify in 4 to 6 hours.*
   - *d. 3 mm thick section of femoral head, on average, will decalcify in 8 to 24 hours.*
7. Check decal completion at regular intervals with Decalcification End Point Set (1051) to deter over-decalcification.
   - *a. See Procedure Note #5.*
8. 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.*
9. Proceed with tissue processing procedure for bone specimens.
10. Trim block and section bone. If trimming or sectioning is impaired due to bone hardness, surface decalcification is recommended.
11. Perform surface decalcification: Soak exposed bone surface side down in Decalcifying Solution, Formic Acid 5%, Aqueous for 15-60 minutes. Rinse block with distilled water to remove corrosive acids and re-section.
    - *a. See Procedure Note #6.*

PROCEDURE NOTES:

1. Other fixatives suitable for bone specimens include: AZF Fixative (1009), B-5 Fixative Modified, Zinc Chloride (1015), Bouin Fluid (1020), Zamboni Fixative (1459) and Zinc Formalin Fixative (1482).
2. Reduce size of a large bone by bisecting bone into smaller pieces, removing excess soft tissue for faster fixation. Maximum bone thickness of 3-5 mm is recommended.
3. 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.
4. Enhance decal with low-speed agitation shaker, rotator or stir plate.
5. Decalcification end-point testing can also be done with specimen radiography. Physical probing of bone is not recommended.
6. 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.