2505 Parview Road • Middleton, WI 53562-2579 • 800-383-7799 • www.newcomersupply.com • newly@newcomersupply.com

Part 1050
Revised July 2015

Decalcifying Solution, HCL/EDTA - Technical Memo

SOLUTION:	**Part 1050A**	**Part 1050A**	**Part 1050C**
Decalcifying Solution, HCL/EDTA	1 liter	6 X 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, HCL/EDTA, combines acid and chelating decalcifying agents, with an added tartrate buffer for prevention of cellular swelling and distortion. This solution provides rapid decalcification, maintains excellent cellular morphology and is suitable for all bone specimens from bone marrow biopsies and disc material (light bone) to femoral head and long bone sections (compact bone). It is not recommended for use when proteoglycan preservation in articular cartilage is important.

METHOD:

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

Technique: Paraffin sections cut at 5 microns on adhesive slides
Solutions: All solutions are manufactured by Newcomer Supply, Inc.

PROCEDURE:

1. Fix bone specimen in fixative of choice, for a length of time sufficient for specimen size and type.
   - a. *See Procedure Notes #2 and #3.*
2. Wash fixed specimen in running tap water for 10 minutes.
3. Submerge fixed bone segment(s) in container of Decalcifying Solution, HCL/EDTA that adequately covers the specimen. A 20:1 ratio is recommended.
   - a. *See Procedure Notes #4 and #5.*
4. Check the specimen regularly for adequate decalcification solution coverage and change the solution at routine intervals during the decalcification process for optimal decalcifying reaction. Decalcification time will vary and is dependent on size and weight of bone.
   - a. *Check light bone samples every 30 to 60 minutes; check compact bone samples every 1 to 2 hours.*
   - b. *Bone marrow or light bone biopsies, on the average, will decalcify in 1 to 2 hours.*
   - c. *A 3 mm thick section of femoral head, on the average, will decalcify in 3 to 4 hours.*
5. Check completion of decalcification with Decalcification End Point Set (1051) regularly to deter over-decalcification and loss of cellular morphology.
   - a. *See Procedure Note #6.*
6. 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.
   - a. *Additional trimming of the decalcified bone can occur at this stage to a size and thickness suitable for tissue processing.*
7. Proceed with laboratory tissue processing procedure for bone specimens.
8. Trim block(s) and section the processed, paraffin embedded bone; if trimming or sectioning is impaired due to bone hardness, surface decalcification is recommended.
   - a. *See Procedure Note #7.*
9. 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.
   - a. *See Procedure Note #8.*

PROCEDURE NOTES:

1. Other fixatives that provide satisfactory results for bone specimens are: AZF Fixative (1009), B-5 Fixative Modified, Zinc Chloride (1015), Bouin Fluid (1020), Zamboni Fixative (1459) and Zinc Formalin Fixative (1482).
2. If possible, reduce the overall size of a larger bone specimen by bisecting or cutting the bone into smaller pieces and remove any excess attached soft tissue or skin for faster fixation. A maximum bone thickness of 3-5 mm is recommended.
3. To ensure optimal staining results, adequate fixation of bone is essential before exposing specimen to decalcification solution.
4. Decal 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.
5. Decalcification can be enhanced with the use of low speed agitation with either a stir bar/stir plate or rotator/shaker.
6. Decalcification end-point testing can also be accomplished through specimen radiography. Physical testing (probing or bending) of the bone is not recommended.
7. Decalcifying Solution, HCL/EDTA 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.
8. 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:

1. Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 338-343.
2. Callis, Gayle and Diane Sterchi. "Decalcification of Bone: Literature Review and Practical Study of Various Decalcifying Agents, Methods, and Their Effects on Bone Histology." *The Journal of Histotechnology* 21.1 (1998): 49-58.
3. 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.
4. Urban, Ken. "Routine Decalcification of Bone." *Laboratory Medicine* 12.4 (1981): 207-212.
5. 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.
6. Modifications developed by Newcomer Supply Laboratory.