

Decalcifying Solution, EDTA/Sucrose - Technical Memo

SOLUTION:

Decalcifying Solution, EDTA/Sucrose

Part 1048B

1 liter

Part 1048C

1 Gallon

Part 1048D

10 Liter Cube

Additionally Needed:

Decalcification End Point Set

Part 1051

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

APPLICATION:

Newcomer Supply Decalcifying Solution, EDTA/Sucrose procedure uses a chelating agent with an added TRIS buffer for gentle bone decalcification. Decalcification rate will be slower but preservation of cellular morphology is excellent and viability of staining for enzymes, immunohistochemistry antigenicity and electron microscopy is maintained. This solution 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, EDTA/Sucrose that adequately covers the specimen. A 20:1 ratio is recommended.
 - a. *See Procedure Notes #4 and #5.*
4. Check the specimen daily for adequate decalcification solution coverage and change the solution regularly during the decalcification process for optimal decalcifying reaction. The process of decalcification with Decalcifying Solution, EDTA/Sucrose can take from 2-14 days, and will be dependent on specimen type, thickness and weight. Larger bones may require an even longer period of decal exposure.
 - a. *See Procedure Note #6.*
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 #7.*
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 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.

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. Decalcification 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. Changing Decalcifying Solution, EDTA/Sucrose Solution at regular intervals during the process ensures the chelating agent has not been depleted by its reaction with calcium.
7. Decalcification end-point testing can also be accomplished through specimen radiography. Physical testing (probing or bending) of the bone is not recommended.

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. Hao, Zhengling, Vicki Kalscheur, and Peter Muir. "Decalcification of Bone for Histochemistry and Immunohistochemistry Procedures." *The Journal of Histotechnology* 25.1 (2002): 33-37.
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.