

## Decalcifying Solution, EDTA/Sucrose - Technical Memo

**SOLUTION:**

Decalcifying Solution, EDTA/Sucrose

**1 Liter**

Part 1048B

**1 Gallon**

Part 1048C

**10 Liter Cube**

Part 1048D

**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 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, EDTA/Sucrose, adequately covering specimen at a 20:1 ratio.
  - a. See Procedure Notes #3 and #4.
5. Check specimen daily for adequate solution coverage. Change solution at least daily to ensure chelating agent is not depleted by its reaction with calcium. Do not add or mix fresh solution with old.
6. Decalcification with Decalcifying Solution, EDTA/Sucrose can take from 2-14 days, dependent on specimen type, thickness and weight. Larger bones may require longer decal exposure.
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.

**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 larger bone by bisecting bone into smaller pieces and remove excess soft tissue and skin 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.

**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.