

Decalcification End Point Set - Technical Memo

SET INCLUDES:Solution A: Ammonium Hydroxide, Aqueous
Solution B: Ammonium Oxalate, Aqueous**Part 1051A**250 ml
250 ml

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

APPLICATION:

Newcomer Supply Decalcification End Point Set uses a chemical testing method for detecting the presence of released calcium and determining the completion point of bone decalcification process. This procedure will avoid over decalcification and limit the decalcifying process to what is needed to maintain a high-quality specimen. Decalcification End Point Set can be used with either acid or chelating decalcifying agents.

METHOD:

Fixation: Fully fixed bone specimen in fixative of choice.

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

PROCEDURE:

1. Submerge fixed and washed bone segment in decalcifying solution of choice, adequately covering specimen at a 20:1 ratio.
2. Check 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 specimen type, thickness and strength of the decalcifying agent used.
4. Check completion of decal with Decalcification End Point Set. Prepare a test aliquot by pipetting and combining in a 20 ml beaker:
 - a. Solution A: Ammonium Hydroxide, Aqueous 5 ml
 - b. Solution B: Ammonium Oxalate, Aqueous 5 ml
 - c. Used decal solution from specimen container 5 ml
 - d. Avoid pipetting up particulates from used solution
5. Mix well; allow test aliquot to stand for 15 minutes.
6. Decalcification is complete if test aliquot is clear.
 - a. Clear indicates solution is calcium free and decalcification has reached an end point stage.
 - b. Proceed to Step #8.
7. Decalcification is incomplete if the test aliquot is cloudy.
 - a. Cloudy indicates presence of calcium oxalate and decalcification is incomplete.
 - b. Transfer specimen into fresh decalcification fluid and continue to repeat Step #3 – Step #6 until decalcification end point stage is reached.
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 NOTE:

1. 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. 341-342.
2. Carson, Freida L., and Christa Hladik Cappellano. *Histotechnology: A Self-instructional Text*. 4th ed. Chicago: ASCP Press, 2015. 48-49.
3. Luna, Lee G. *Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts*. Gaithersburg, MD: American Histolabs, 1992. 115-118.
4. Modifications developed by Newcomer Supply Laboratory.