

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 calcium released from bone and determining the completion point of the bone decalcification process. This procedure will assist to avoid over decalcification, a common cause of loss of specimen morphology 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 thoroughly washed bone segment(s) in container of decalcifying solution of choice that adequately covers the specimen. A 20:1 ratio is recommended.
2. Check the specimen for adequate decalcification solution coverage and change the solution regularly during the decalcification process for optimal decalcifying reaction. The process of decalcification will be dependent upon specimen type, thickness and strength of the decalcifying agent used.
3. Check completion of decalcification with Decalcification End Point Set. Prepare a test aliquot by pipetting and combining in a clean 20 ml beaker or flask:
 - a. *Solution A: Ammonium Hydroxide, Aqueous 5 ml*
 - b. *Solution B: Ammonium Oxalate, Aqueous 5 ml*
 - c. *Used decal solution from specimen container bottom 5 ml*
 - d. *Avoid picking up decalcification solution particulates from the specimen container into the pipette.*
4. Mix well; allow test aliquot to stand for 15 minutes.
5. Decalcification is complete if the test aliquot is clear or colorless.
 - a. *Clear/colorless solution indicates the solution is calcium free and decalcification has reached an end point stage.*
 - b. *Proceed to Step #7.*
6. Decalcification is incomplete if the test aliquot is cloudy or turbid.
 - a. *Cloudy/turbid solution indicates the presence of precipitated calcium oxalate and decalcification is incomplete.*
 - b. *Transfer the specimen into fresh decalcification fluid and continue to repeat Step #3 – Step #6 as needed to reach a decalcification end point stage.*
7. 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.*
8. Proceed with laboratory tissue processing procedure for bone specimens.

PROCEDURE NOTES:

1. 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. 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.