



DehCL+

Suggested Methodology for 0.2 cm Bone Marrow Biopsies

Processing time: less than 1 hour

DehCL^{®+} may be used to process all types of calcified histological specimens.

This methodology is meant to be used as a guideline in establishing a protocol. Larger, denser bone will take longer to decalcify than the tissue referred to here. Fixation and decalcification times will need to be increased for denser, larger bone.

Consistency

It is easier to achieve consistent results by preparing uniform specimens for decalcification. For larger bone, the use of a bone saw is highly recommended. If decalcification time exceeds 24 hours, it is best to replace the decalcifying solution with fresh solution daily.

Fixation

Fix tissue in 10% Neutral Buffered Formalin or other suitable tissue fixative such as Bouins, B5, or Zinc Formalin. The volume of fixative solution should be 20 times the tissue volume.

Decalcification

Briefly rinse fixed tissue in running water. Immerse rinsed tissue in a volume of **DehCL+** equal to at least 20 times the volume of tissue.

Agitation and Heat

Gentle agitation will greatly enhance decalcification quality and reduce decalcification time. Gentle application of heat will also reduce decalcification times.

Endpoint

Check tissue every half hour. Probing tissue to determine flexibility is the simplest and most common method for determining endpoint. Furthermore, if the tissue begins to float in solution, decalcification is usually complete although tissue will not necessarily float when it is decalcified. For a more accurate endpoint determination, please refer to the ammonium oxalate turbidity test.

Processing

After decalcification is complete, rinse tissue briefly in deionized water before placing tissue in the processor. This will enhance staining after processing. The use of deionized water eliminates the possible contamination of tissue by chemicals which may be present in municipal tap water.

Please Note: *If you plan to stain the section with a Potassium Ferrocyanide / Hcl stain, a minimum 10-minute rinse is recommended.*

Sectioning / Surface Decalcifying

To surface decalcify embedded bone, place a small dish of **DehCL+** on ice. Place the face of the block in the dish for 5-10 minutes. Rinse the block in cold water. Icing tends to make the block harder and the water shed tends to soften the tissue face. Icing will greatly reduce the amount of chattering, especially in large blocks.

If you do not achieve the results you are looking for, please contact us.

