Decal®

Suggested Methodology

0.2 cm Bone Marrow Biopsies
Processing time, 20 minutes - 1 hour.

Decal® may be used to process all types of calcified histological specimens.
This methodology is only meant to be used as a guideline in establishing a protocol. Larger, more dense bone will take longer to decalcify than the tissue referred to in this methodology. Fixation and decalcification times will need to be increased for denser, larger bone.

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 ten times the tissue volume.

Decalcification
Rinse fixed tissue in running water for at least 3 minutes. Immerse tissue in a volume of Decal® equal to at least 10 times the tissue volume.

Endpoint
Check tissue every half hour. If tissue is floating or flexible, decalcification is complete. However, tissue will not necessarily float when it is decalcified. For a more accurate endpoint determination, approximately 5 mL of the used decalcifying fluid should be made neutral to litmus paper with concentrated ammonium hydroxide. Then approximately 5mL of saturated ammonium oxalate solution is added. The resulting solution is mixed well and allowed to stand for 30 minutes; a persistent turbidity (calcium oxalate) indicates the presence of calcium. (*Carson, Freida L., Histotechnology, A Self-Instructional Text, ASCP Press, 1990, P 40)*

Processing
To enhance staining after decalcification, place tiusse in Cal-Arrest for approximately 10 minutes. Then rinse tissue in running water for approximately 3 minutes. Handle as ordinary tissue. The use of deionized water eliminates the possible contamination of tissue by such chemicals as chlorine, sulphur, magnesium, lead and innumerable other dissolved and undissolved solids 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
If chipping or crunching occurs on the microtome, paraffin embedded sections may be surface decalcified by placing the face of the block in a dish of Decal® for 5-10 minutes. Rinse the block in cold water and then ice the block for 3-5 minutes. 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.

Please note: For the most consistent results, do not attempt to reuse Decal®. As the decalcification process takes place, the strength of Decal® will diminish. Therefore any tissue place in a “used” Decal® solution will fix and decalcify more slowly than a similar section placed in a fresh solution.

If you do not achieve the results you are looking for, please contact our technical support department at 1-800-428-5856 or via email at: Sales@decal-bone.com

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