

Immuno-4tm

Suggested Protocol for 0.2 cm Bone Marrow Biopsies

Processing time: 2-4 hours

Immuno-4tm may be used to process all types of calcified histological specimens.

This protocol is meant to be used as a guideline in establishing a protocol. Larger, denser bone will take longer to decalcify than the tissue size referenced 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

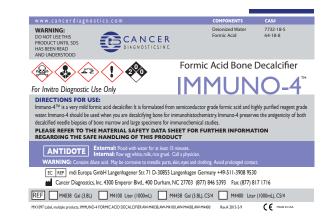
Fix tissue in 10% Neutral Buffered Formalin or other suitable tissue fixative such as Bouins, B5, B5-SUB (CDI) or Zinc Formalin. The volume of fixative solution should be 20 times the tissue volume.

Decalcification

Briefly rinse fixed tissue in running water. Immerse rinsed tissue in a volume of **Immuno-4**tm 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 1/2 hour. Probing tissue to determine flexibility is the simplest and most common method for determining endpoint. (Additionally, 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

Rinse tissue briefly in deionized water after decalcification is complete before placing tissue in the processor as this will enhance staining after processing. The use of deionized water eliminates the possible contamination of tissue by trace chemicals and contaminants that are commonly present in municipal tap water.

Please Note: When staining 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 Immuno-4tm 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 rinse 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 our technical support department at 1-877-846-5393x702 or via email at: info@cancerdiagnostics.com