Immunocal®

Suggested Methodology

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

Fixation

Fix section in 10% buffered formalin, B5, Zinc Formalin, or other suitable fixative. Be sure to fix section in a volume of fixative equal to at least 10 times the size of section.

Decalcification

When tissue is fixed, wash section in running deionized water for at least 3 minutes. (The use of deionized water will eliminate the possibility that contaminants such as cholrine, lead, copper, magnesium and phosphates will be absorbed by the tissue and interfere with staining.) Immerse rinsed section in a volume of Immunocal equal to at least 10 times the size of section. If necessary, replace solution every 24 hours. Decalcification times will vary depending on the type of specimen (bone marrows will decalcify more quickly than femoral heads) and the nature of the specimen (a specimen from a 20 year old male will probably decacify more quickly than comparable tissue from a 75 year old female). It is best to establish your own base time and develop protocol from there.

Endpoint

Endpoint may be determined through one of the following methods:

a. Radiography is the most reliable method of determining complete decalcification of tissue. However, this method cannot be used if the tissue has been fixed in a "metallic fixative" such as Zenkers or B-5. (*Carson, Freida L., Histotechnology, A Self-Instructional Text, ASCP Press, 1990, P 40)*

b. Ammonium Oxalate Method: Add concentrated ammonium hydroxide to approximately 5 mL of the used decalcifying fluid until the solution is neutral (6.7-7.2 pH @ 25℃). Then add to this solution approximately 5mL of saturated ammonium oxalate. Mix well and allow to stand for 30 minutes; a persistent turbidity (calcium oxalate) indicates the presence of calcium.

c. If the tissue is flexible to the touch of a pin or if it is floating in the decalcifying solution, most or all of the calcium has been extracted. This is by far the easiest and most common method of determining endpoint. Unfortunately it is also the least reliable.

Neutralization

Once endpoint has been determined, the section may be placed in Cal-Arrest, a neutralizing solution that performs two functions. First, it neutralizes the pH of the tissue which enhances embedding and staining characteristics. Second, it stops decalcification so that the tissue does not become over decalcified. The tissue should remain in this solution for 2-3 minutes.

Processing

Before processing, rinse tissue in running water for at least 10 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.

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 **Immunocal** 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.

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:
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