



# Immuno-4<sup>tm</sup>

## *Suggested Protocol for 0.2 cm Bone Marrow Biopsies*

Processing time: 2-4 hours

Immuno-4<sup>tm</sup> 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<sup>tm</sup> 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.

www.cancerdiagnostics.com		COMPONENTS	CAS#
<b>WARNING:</b> DO NOT USE THIS PRODUCT UNTIL SDS HAS BEEN READ AND UNDERSTOOD		Deionized Water Formic Acid	7732-18-5 64-18-8
		<b>Formic Acid Bone Decalcifier</b> <b>IMMUNO-4<sup>TM</sup></b>	
For <i>In Vitro</i> Diagnostic Use Only		<b>DIRECTIONS FOR USE:</b> Immuno-4 <sup>TM</sup> is a very mild formic acid decalcifier. It is formulated from semiconductor grade formic acid and highly purified reagent grade water. Immuno-4 should be used when you are decalcifying bone for immunohistochemistry. Immuno-4 preserves the antigenicity of both decalcified needle biopsies of bone marrow and large specimens for immunochemical studies. <b>PLEASE REFER TO THE MATERIAL SAFETY DATA SHEET FOR FURTHER INFORMATION REGARDING THE SAFE HANDLING OF THIS PRODUCT</b>	
<b>ANTIDOTE</b>		External: Flood with water for at least 15 minutes. Internal: Raw egg white, milk, rice gruel. Call a physician.	
<b>WARNING:</b> Contains dilute acid. May be corrosive to metallic parts, skin, eyes and clothing. Avoid prolonged contact.			
EC REP mdi Europa GmbH Langenhagener Str. 71 D-30855 Langenhagen Germany +49-511-3908 9530 Cancer Diagnostics, Inc. 4300 Emperor Blvd., 400 Durham, NC 27703 (877) 846 5393 Fax: (877) 817 1716			
REF <input type="checkbox"/> M4038 Gal (3.8L) <input type="checkbox"/> M4100 Liter (1000mL) <input type="checkbox"/> M4438 Gal (3.8L), CS/4 <input type="checkbox"/> M4400 Liter (1000mL), CS/4			
PKX1097 Label, multiple products, IMMUNO-4 FORMIC ACID DECALCIFIER, AVX-M4038, AVX-M4100, AVX-M4438, AVX-M4400 Rev A 2015-2-9  MADE IN USA			



### ***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-4™** 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](mailto:info@cancerdiagnostics.com)***

