



RegularCal•Immuno™

Intended Use

RegularCal•Immuno™ is designed for decalcification of all bony or ossified tissues to be studied by sophisticated immunochemical techniques. It can be used on needle biopsies of bone marrow and on large specimens of bone. RegularCal•Immuno™ preserves with excellence the antigenicity of decalcified tissues for immunochemical studies.

General Information

RegularCal•Immuno™ is a decalcifying solution that is formulated to accomplish decalcification and produce excellent histological and immunochemical results. Needle biopsies and other small biopsies will decalcify in 12-24 hours. Bony cassettes-size tissues 2-3 millimeters in thickness will decalcify in 24-48 hours. Small tissues left in RegularCal•Immuno™ for days and weeks maintain the ability to display excellent H&E stained sections. This decalcifying solution is compatible with all fixatives, and all special stains perform well following use with RegularCal•Immuno™.

Packaging

Catalog #	Volume
6078	1 pint
6080	1 qt
6085	1 gal

Fixation Procedure

Fixation of larger specimens of bone is usually in 10% Neutral Buffered Formalin. For bone marrow biopsies, special fixatives are often used. These include B•Plus™ designed particularly for bone marrow biopsies. Fixatives containing mercury also perform well. The decalcification procedure for all fixatives is the same; consequently, we will describe the fixation procedure only for 10% Neutral Buffered Formalin.

1. The bone or ossified biopsies or tissues should be added directly to the 10% Neutral Buffered Formalin. No other dilution or addition of other agents is necessary before use.
2. Small biopsies, such as bone marrow biopsies, should be fixed at least 3 hours prior to processing. Cassette-size sections from large bony specimens, such as femoral heads, are best fixed 10-12

hours, although fixation for 46 hours is often sufficient. Over-fixation is not a problem; however, tissues should generally not be fixed longer than one to two weeks.

3. No washing of tissues after fixation is necessary.
4. The fixed tissues should be processed by the standard processing schedules that may vary from one hour to 12 hours. Recommended BBC tissue processing schedules are available on request.
5. The schedule for staining tissues fixed with 10% Neutral Buffered Formalin is similar to the schedule published in standard texts of histology. Our suggested staining schedule follows.
6. Disposal 10% Neutral Buffered Formalin should be the same as that used for fixatives containing formaldehyde. Consult your local waste water disposal authority for specific instructions.

Staining Procedure

BBC RECOMMENDED AUTOMATED AND MANUAL HISTOLOGY STAINING PROCEDURE FOR HARRIS HEMATOXYLIN AND EOSIN

*Initially deparaffinize tissue sections with BBC S3•Histo™ or Xylene

Step *	Solution	Time
1.	100% Alcohol.....	20 seconds
2.	100% Alcohol.....	20 seconds
3.	95% Alcohol.....	20 seconds
4.	95% Alcohol.....	20 seconds
5.	70% Alcohol.....	20 seconds
6.	Running H ₂ O Wash.....	30 seconds
7.	BBC Harris Hematoxylin	4-5 minutes
8.	Running H ₂ O Wash.....	1 minute
9.	BBC Acid Wash•Histo™	1 minute
	or BBC Acid Alcohol•Histo™	2-3 seconds
10.	Running H ₂ O Wash.....	1 minute
11.	BBC Blueing Solution•Histo™	15 seconds
12.	Running H ₂ O Wash	1 minute
13.	70% Alcohol.....	30 seconds
14.	BBC Special Eosin I™ or II™, or Eosin Y, or Eosin Y with Phloxine B.....	1 minute
15.	BBC S2•Histo™	20 seconds
17.	BBC S2•Histo™	20 seconds
18.	BBC S2•Histo™	20 seconds
19.	BBC S2•Histo™	20 seconds
20.	BBC S2•Histo™	20 seconds
21.	BBC S3•Histo™ or Xylene.....	20 seconds
22.	BBC S3•Histo™ or Xylene.....	30 seconds
23.	BBC S3•Histo™ or Xylene.....	30 seconds
24.	Mount and coverslip with Optic Mount I™ or an appropriate mounting medium.	

Note: Each of these reagents can be intermixed and used with other staining sequences and other manufacturer's reagents.