



# RapidCal•Immuno™

## Intended Use

RapidCal•Immuno™ is a rapid decalcifier designed to preserve immunogenicity at a maximum while accomplishing rapid decalcification. It is designed specifically for bone marrow biopsies and small diagnostic portions of bone, although it can also be used effectively on large fragments of bone.

## General Information

RapidCal•Immuno™ is an acid decalcifier formulated to preserve immunogenicity of epitopes at as high a sensitivity of detection as possible. With small biopsies, sensitivity of detection of antigens is preserved approaching 100% for most all epitopes. Following decalcification of bone marrows for 1-2 hours, the sensitivity of detection of hematopoietic markers and B and T cell markers are preserved nearing the 100% level. Sensitivity of detection of intermediate filaments and cyto skeletal proteins and epithelial cell markers are similarly maintained.

RapidCal•Immuno™ is intended to be used primarily on fixed bone core biopsies and small diagnostic fragments of bone. It can also be used on cassette-sized pieces of bone. The time of decalcification of an 11-gauge bone core biopsy is 1 hour; that of an 8-gauge biopsy is approximately 90 minutes.

Fixation of tissues to be decalcified with RapidCal•Immuno™ can be accomplished with any fixative. However, the sensitivity of most commercial antibodies is set for formaldehyde fixation. Following fixation with neutral buffer formalin, decalcified tissues should be stained at the routine times used for non-decalcified tissues. Tissues fixed in zinc formalin prior to decalcification may require a 1 to 2 fold lower dilution of antibody for equivalent sensitivity.

## Packaging

Catalog #	Volume
6086	1 pint
6089	1 gal

## Fixation Procedure

Bone marrow biopsies should be fixed in B•Plus™ or 10% Neutral Buffered Formalin. The best histological preservation is accomplished with fixation in B•Plus™. Fixation in B•Plus™ can be performed in 2-3 hours; fixation in 10% Neutral Buffered Formalin requires a minimum of 8-12 hours.

The biopsies or tissues should be added directly to B•Plus™ or 10% Neutral Buffered Formalin. No other dilution or addition of other agents is necessary before use.

- Small biopsies, such as bone marrow biopsies, should be fixed in B•Plus™ for at least 3 hours prior to processing. When using 10% Neutral Buffered Formalin, fix for 8-12 hours. Over-fixation is not a problem; however, tissues should generally not be fixed longer than one to two weeks.
- Place bone core biopsies in RapidCal•Immuno™
  - 11-gauge biopsy for 1 hour
  - 8-gauge biopsy for 90-120 minutes
  - 2-3 mm slices of bone for 3-4 hours.
- Wash decalcified bone cores or tissue blocks for 10 minutes in running tap water.
- Place the washed tissues on tissue processor for routine processing and infiltration with paraffin.
- The schedule for staining tissues fixed with 10% Neutral Buffered Formalin is the similar to the schedule published in standard texts of histology. Our suggested staining schedule follows.

## 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

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 <sub>2</sub> O Wash .....	30 seconds
7. BBC Harris Hematoxylin .....	4-5 minutes
8. Running H <sub>2</sub> O Wash .....	1 minute
9. BBC Acid Wash•Histo™ .....	1 minute
or BBC Acid Alcohol•Histo™ ....	2-3 seconds
10. Running H <sub>2</sub> O Wash .....	1 minute
11. BBC Blueing Solution•Histo™ .....	15 seconds
12. Running H <sub>2</sub> 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.