



# Regular•Cal™

## Intended Use

RegularCal™ is designed to use for decalcification of all bony or ossified tissues. It can be used on needle biopsies of bone marrow and on large specimens of bone. RegularCal™ is the best type of acid decalcifier to use on large specimens of bone and on cassette-size sections of bone. RapidCal™ also preserves with excellence the antigenicity of decalcified tissues for immunochemical studies.

## General Information

RegularCal™ is a decalcifying solution that is formulated to accomplish relatively rapid decalcification and preserve excellent histological and immunochemical results. Needle biopsies and other small biopsies will decalcify in 4-6 hours. Bony cassettes-size tissues 2-3 millimeters in thickness will decalcify in 8-12 hours. Small tissues left in RegularCal™ overnight 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™. If more rapid decalcification is desired, RapidCal™ can be used.

## Packaging

Catalog #	Volume
6056	1 pint
6060	1 quart
6070	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. The decalcification procedure for all fixatives is the same; consequently, we will describe the fixation procedure only for 10% Neutral Buffered Formalin.

1. The 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 specimen, such as femoral heads, are best fixed 10-12 hours, although fixation for 4-6 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. Standard 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

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.