



## RapidCal™

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### Intended Use

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RapidCal™ is a decalcifying solution formulated to decalcify bone rapidly. It is gentle enough to be used as the routine rapid decalcifying solution for bone marrow biopsies. 11 gauge biopsies can be decalcified in 45 minutes. The usual bone marrow biopsy obtained from an 8-gauge Jamshidi needle requires about 60-90 minutes to decalcify. Smaller needle biopsies require less time for decalcification. RapidCal™ is compatible with all fixatives including mercury-containing fixatives such as B-5, B-Plus with Mercuric Chloride, Zenker's and Helley's. It is also compatible with all fixatives, including B-Plus without Mercuric Chloride and 10% Neutral Buffered Formalin. All special stains including reticulin stains perform well following use of RapidCal™. RapidCal™ is the rapid decalcifying solution of choice for bone marrow biopsies to be interpreted by routine light microscopy. If however, immunochemical studies are desired, RegularCal™ or RegularCal-Immuno™ is a better choice as a decalcifier.

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### General Information

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The choice of acid decalcifiers for bone specimens is dependent upon the time of decalcification available, the histologic requirements for the appearance of the stained sections and the need for special studies, such as immunochemical stains. Rapid decalcification is accomplished best by a decalcifier containing HCl; yet tissues can be exposed to HCl decalcifiers only up to 48 hours without suffering compromised H & E staining. If longer periods of decalcification can be tolerated, decalcifiers containing formic acid produce improved H & E staining results. If immunochemical studies are to be performed, a slower decalcifier such as RegularCal™ or RegularCal-Immuno™ should be used. For enzyme studies, particular formulations of decalcifiers are available.

Bone marrow biopsies require special consideration regarding the choice of decalcifiers. For rapid decalcification of a bone marrow biopsy (e.g.  $\leq 90$  min.), decalcifiers containing a mineral acid, such as HCl, can be used. These decalcifiers must be used with caution because they rapidly hydrolyze proteins during decalcification. If 4-5 hours is tolerable for decalcification, slower decalcifiers such as RegularCal™ should be used, since they generally produce excellent H & E results and improved immunochemical studies.

Larger portions of bone no larger than 2.0 cm x 1.5 cm x 0.3 cm can be decalcified by rapid decalcifiers, such as RapidCal™. The histology defined by H & E staining is generally better with slower decalcifiers. Rapid decalcifiers are also limited in that tissues exposed to these for more than 48 hours will suffer significant histologic compromise. In contrast, tissues can be exposed to RegularCal™ for up to 10 days without significant histologic damage. Larger portions of bone must consequently be decalcified with slower decalcifiers, such as RegularCal™.

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### Packaging

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Catalog #	Volume
6029	1 pint
6030	1 qt
6040	1 gal
6043	1 gal cube

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### Fixation Procedure

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Fixation of larger specimens of bone is usually in 10% Neutral Buffered Formalin. For bone marrow biopsies, special fixatives such as B-Plus™ 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. The fixation procedure for B-Plus™ is available on request.

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 the 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 wastewater disposal authority for specific instructions.

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### Staining Procedure

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See next page

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## Staining Procedure

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

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

<u>Solution</u>	<u>Time</u>
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
<b>or</b> 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™, <b>or</b> Eosin Y, <b>or</b> 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.