

# Acid Wash•Histo™

Catalog # 3810

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## Intended Use

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Acid Wash•Histo™ is designed to differentiate hematoxylin in regressive H&E staining procedures. It produces ideal differentiation of nuclear and non-nuclear structures. Nuclei show precise nuclear membranes, and non-nuclear structures stain optimally with eosin. Mucin is not stained.

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## General Information

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Acid Wash•Histo™ is a differentiator of hematoxylin in regressive H&E staining procedures. Nuclei demonstrate precisely stained nuclear chromatin and parachromatin, nuclear membranes are distinct, and nucleoli are discrete. Non-nuclear structures show excellent staining by eosin with the ideal variations of staining of erythrocytes, smooth muscle, and connective tissues. Mucin is not stained with hematoxylin following differentiation with Acid Wash•Histo™. This is the optimum differentiator for use with BBC Harris Hematoxylin.

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## Packaging

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| Catalog # | Volume  |
|-----------|---------|
| 3814      | 1 qt    |
| 3816      | 4x1 gal |

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## Fixation Procedure

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Acid Wash•Histo™ is a differentiator of hematoxylin used with regressive H&E staining procedures. It works ideally with tissues fixed in virtually any type of fixative. The time of differentiation of Acid Wash•Histo™ should not vary regardless of the fixative used. Acid Wash•Histo™ is designed to work particularly well following the fixation of tissues in 10% neutral buffered formalin and other routine fixatives.

Because Acid Wash•Histo™ performs well for differentiation of hematoxylin following any fixative; we will describe only the fixation procedure of 10% neutral buffered formalin.

10% Neutral Buffered Formalin is a non-coagulative additive fixative. The buffering capacity of our 10% Neutral Buffered Formalin enhances staining by H&E and immunohistochemistry.

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. Tissue blocks from large tissues, such as lymph nodes or spleen or breast or colon, 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 schedules published in standard texts of histology. Our suggested schedule follows.
6. Disposal of 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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BBC RECOMMENDED AUTOMATED AND MANUAL HISTOLOGY STAINING PROCEDURE FOR HARRIS HEMATOXYLIN AND EOSIN

\*Initially deparaffinize tissue sections with BBC S1™ 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 <sub>2</sub> O Wash .....                                           | 30 seconds  |
| 7.     | BBC Harris Hematoxylin .....                                                  | 3-5 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 dips    |
| 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<br>Eosin Y, or Eosin Y<br>w/ Phloxine B ..... | 45 seconds  |
| 15.    | BBC S2•Histo™ .....                                                           | 20 seconds  |
| 16.    | 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 S3™ or Xylene .....                                                       | 20 seconds  |
| 21.    | BBC S3™ or Xylene .....                                                       | 30 seconds  |
| 22.    | BBC S3™ or Xylene .....                                                       | 30 seconds  |
| 23.    | Mount and coverslip with Optic Mount I™ or an<br>appropriate mounting medium. |             |

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