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# Rapid Hematoxylin & Eosin (H&E) Stain for Frozen Sections Technical Memo

| **SOLUTIONS:** | **500 ml** | **1 Liter** | **1 Gallon** |
|---|---|---|---|
| Hematoxylin Stain, Harris Modified | Part 1201A | Part 1201B | Part 1201C |
| Eosin Y Working Solution | Part 1072A | Part 1072B | Part 1072C |

Additionally Needed For H&E Frozen Section Staining:

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|---|---|
| TruBond 380 Adhesive Microscope Slides | Part 5080 |
| EasyDip™ Slide Staining Jar | Part 5300 |
| EasyDip™ Slide Staining Rack | Part 5300RK |
| Formalin 10%, Phosphate Buffered | Part 1090 |
| Alcohol, Ethyl Denatured, 95% | Part 10842 |
| Alcohol, Ethyl Denatured, 100% | Part 10841 |
| Xylene, ACS | Part 1445 |

***For storage requirements and expiration date refer to individual bottle labels.***

## APPLICATION:

Newcomer Supply Rapid Hematoxylin & Eosin (H&E) Stain for Frozen Sections is used for quick microscopic analysis of intraoperative tissue specimens and other cryosection applications such as; enzyme histochemistry, Moh's surgery and demonstration of soluble substances.

Hematoxylin Stain, Harris Modified is a ready to use high quality hematoxylin that does not require filtering and is completely mercury-free. This modified Harris formulation contains glacial acetic acid for more precise and selective nuclear staining and ethylene glycol to increase solution stability and reduce surface precipitate.

Eosin Y Working Solution is a ready-to-use counterstain with the ability to distinguish between the cytoplasm of different types of cells by staining cytoplasmic components differing shades and intensities of pink to red.

**Quality Control:** Since hematoxylin and eosin staining is the foundation of the diagnostic process, maintaining quality is of critical importance. Change staining solutions on a regular basis according to laboratory protocol. Procedures may vary between laboratories depending upon volume of slides, chemical hygiene and solution integrity.

## METHOD:

**Technique:** Frozen sections cut at 3-6 microns on adhesive slides.
**Solutions:** All solutions are manufactured by Newcomer Supply, Inc.

## STAINING PROCEDURE:

1. Immediately fix frozen sections in 95% ethyl alcohol for 15 seconds.
    a. *See Procedure Note #1.*
2. Transfer to Formalin 10%, Phosphate Buffered (1090) for 10 dips.
    a. *See Procedure Notes #2, #3 and #4.*
3. Rinse well in distilled water; 10 dips.
4. Stain with Hematoxylin Stain, Harris Modified for 30 seconds.
5. Wash well in two changes of distilled water; 10 dips each.
6. Place in 95% ethyl alcohol for 10 dips.
7. Counterstain in Eosin Y Working Solution for 15 seconds.
8. Dehydrate in two changes of 95% ethyl alcohol and two changes of 100% ethyl alcohol, 10 dips each. Clear in two changes of xylene, 10 dips each; coverslip with compatible mounting medium.

## RESULTS:

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|---|---|
| Nuclei | Blue |
| Erythrocytes and eosinophilic granules | Bright pink to red |
| Cytoplasm and other tissue elements | Various shades of pink |

## PROCEDURE NOTES:

1. To maintain preservation of tissue morphology, do not allow frozen sections to air-dry.
2. Other methods of acceptable frozen section fixation include; Formaldehyde 37-40%, ACS (1089) and Acetone, ACS (10014).
3. Drain slides after each step to prevent solution carry over.
4. Do not allow sections to dry out at any point during staining procedure.
5. If using a xylene substitute, closely follow the manufacturer's recommendation for clearing step.

## REFERENCES:

1. Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 127.
2. Carson, Freida L., and Christa Hladik Cappellano. *Histotechnology: A Self-instructional Text*. 4th ed. Chicago: ASCP Press, 2015. 120-121.
3. Modifications developed by Newcomer Supply Laboratory.