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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:

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:

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.