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Hematoxylin & Eosin (H&E) Regressive Stain - Technical Memo

SOLUTIONS:	**500 ml**	**1 Liter**	**1 Gallon**
Hematoxylin Stain, Harris	Part 12013A	Part 12013B	Part 12013C
Eosin Y Working Solution	Part 1072A	Part 1072B	Part 1072C

Additionally Needed For H&E Staining:				
Hematoxylin & Eosin (H&E) Control Slides	Part 4278			
Xylene, ACS	Part 1445			
Alcohol, Ethyl Denatured, 100%	Part 10841			
Alcohol, Ethyl Denatured, 95%	Part 10842			
Acid Alcohol 1%	Part 10011			
Lithium Carbonate, Saturated Aqueous	Part 12215	or	Scott Tap Water Substitute	Part 1380
Alcohol, Ethyl Denatured, 70%	Part 10844			

For storage requirements and expiration date refer to individual product labels.

APPLICATION:

Newcomer Supply Hematoxylin & Eosin (H&E) Regressive Stain is used for screening specimens in anatomic pathology, as well as for research, smears, touch preps and other applications. In regressive staining, tissue sections are deliberately overstained then further differentiated with dilute acid until the optimal endpoint is reached.

Hematoxylin Stain, Harris is a ready to use high quality regressive hematoxylin that does not require filtering, is completely mercury-free and does not contain glacial acetic acid or ethylene glycol.

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 will vary between laboratories depending upon volume of slides, automation vs manual staining, chemical hygiene and solution integrity. The longevity of hematoxylin and eosin depend upon these factors and stain quality should be regularly screened with the use of an H&E control slide

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 5 microns
Solutions: All solutions are manufactured by Newcomer Supply, Inc.

STAINING PROCEDURE:

1. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
    a. *See Procedure Notes #1 and #2.*
2. Stain with Hematoxylin Stain, Harris, 1 to 5 minutes, depending on preference of nuclear stain intensity.
3. Wash well in three changes of tap water.
4. Differentiate quickly in Acid Alcohol 1% (10011).
    a. *See Procedure Note #3.*
5. Rinse immediately in three changes of tap water.
6. Blue slides in Lithium Carbonate, Saturated Aqueous (12215) or Scott Tap Water Substitute (1380) for 10 dips.
7. Wash in three changes of tap; rinse in distilled water.
8. Drain excess water from rack/slides; proceed to 70% alcohol for 10 dips.
9. Counterstain in Eosin Y Working Solution for 30 seconds to 3 minutes, depending on preference of intensity.
10. Dehydrate in two changes of 95% ethyl alcohol for 1 minute each and two changes of 100% ethyl alcohol, 10 dips each. Clear in three 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. Drain staining rack/slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during staining procedure.
3. Differentiate for a length of time to suit preference of nuclear stain intensity. Check slides microscopically to assure hematoxylin intensity is satisfactory. Nuclei should be distinct and the background very light to colorless.
4. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 123-126.
2. Carson, Frieda, *Histotechnology: A Self-Instructional Text*. 2nd ed. Chicago: ASCP Press, 1997. 91-96.
3. Luna, Lee G. *Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts*. Gaitheresburg, MD: American Histolabs, 1992. 86-87, 91-92.
4. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 143-144, 153-154.
5. Modifications developed by Newcomer Supply Laboratory.

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