

Hematoxylin Stain, Harris - Technical Memo

STAIN SOLUTION:

Hematoxylin Stain, Harris	Part 12013A 500ml	Part 12013B 1 Liter	Part 12013C 1 Gallon
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Additionally Needed For H&E Staining:

Hematoxylin and 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
Alcohol, Ethyl Denatured, 70%	Part 10844		Part 1380
Eosin Y Working Solution	Part 1072	or	Eosin-Phloxine Stain Set
			Part 1082

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

APPLICATION:

Newcomer Supply 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. This Harris formulation is designed for either manual or automated staining.

The routine hematoxylin and eosin (H&E) stain is used for screening specimens in anatomic pathology, as well as for research, smears, touch preps and other applications. Its two primary coloring agents stain all cellular material including nuclei (blue), and cytoplasmic elements (pink-red). Popularity of this stain is due, in large measure, to its simplicity, ability to clearly demonstrate a wide variety of different tissue components, dependability, repeatability, and speed of use.

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 depends 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.

H&E STAINING PROCEDURE WITH HEMATOXYLIN, HARRIS:

- 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.
 - See Procedure Notes #1 and #2.
- Stain with Hematoxylin Stain, Harris, 1 to 5 minutes, depending on preference of nuclear stain intensity.
- Wash well in three changes of tap water.
- Differentiate quickly in Acid Alcohol 1%.
 - See Procedure Note #3.
- Rinse immediately in three changes of tap water.
- Blue slides in Lithium Carbonate, Saturated Aqueous (12215) or Scott Tap Water Substitute (1380) for 10 dips.
- Wash in three changes of tap; rinse in distilled water.
- Drain excess water from rack/slides; proceed to 70% alcohol for 10 dips.

- Counterstain in Eosin Y Working Solution (1072) or prepared Eosin-Phloxine Working Solution (1082) for 30 seconds to 3 minutes, depending on preference of intensity.
- 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:

- Drain staining rack/slides after each step to prevent solution carry over.
- Do not allow sections to dry out at any point during staining procedure.
- 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.
- To minimize oxidation and extend shelf life, store hematoxylin at room temperature in tightly capped container away from direct light.
- If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

- Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 123-125.
- Carson, Freida L., and Christa Hladik Cappellano. *Histotechnology: A Self-instructional Text*. 4th ed. Chicago: ASCP Press, 2015. 113, 118-120.
- Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 142-144, 153-154.
- Modifications developed by Newcomer Supply Laboratory.