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Part 1180
Revised July 2015

Hematoxylin Stain, Gill - Technical Memo

STAIN SOLUTION:	**500 ml**		**1 Gallon**	
Hematoxylin Stain, Gill I (single strength)	Part 1180A		Part 1180C	
Hematoxylin Stain, Gill II (double strength)	Part 1180D		Part 1180F	
Hematoxylin Stain Gill III (triple strength)	Part 1180G		Part 1180I	
Additionally Needed For H&E Staining:				
Xylene, ACS	Part 1445			
Alcohol, Ethyl Denatured, 100%	Part 10841			
Alcohol, Ethyl Denatured, 95%	Part 10842			
Lithium Carbonate, Saturated Aqueous	Part 12215	or	Scott Tap Water Substitute	Part 1380
Alcohol, Ethyl Denatured, 70%	Part 10844			
Eosin Y Working Solution	Part 1072	or	Eosin-Phloxine Stain Set	Part 1082

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

APPLICATION:

Newcomer Supply Hematoxylin Stain, Gill, is a ready to use progressive nuclear stain available in three different strengths for cytology and histology applications. Due to the progressive staining nature of Gill hematoxylin, over-staining is less likely and an acid alcohol differentiation step is not required in the staining process. Gill hematoxylin does not require filtering prior to use but if using any Gill hematoxylin for cytology specimen staining, filtering before each use is highly suggested to prevent solution contamination.

Gill I is termed “single strength” and is ideal for both gynecological and non-gynecological cytology preparations. Gill II, noted as “double strength”, is used as a counterstain in immunohistochemistry (IHC) procedures, a frozen section nuclear stain, a routine hematoxylin and eosin (H&E) stain and for more intense cytology staining. Gill III is “triple strength” and is used primarily for tissue sections when a darker nuclear stain is preferred with shorter staining time. Goblet cells will be stained by Gill hematoxylin but not by other hematoxylin solutions.

To minimize oxidation and extend shelf life, store hematoxylin in a tightly capped container and keep stock solutions in the dark at room temperature.

METHOD:

For Histology Specimens
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 STAIN, GILL:

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, Gill of choice. Staining time may vary depending upon the solution strength and intended purpose.
    a. *For recommendations: See Procedure Note #3.*
3. Wash well in three changes of tap water.
4. Blue slides in Lithium Carbonate, Saturated Aqueous (12215) or Scott Tap Water Substitute (1380) for 10 dips.
5. Wash in three changes of tap water; rinse in distilled water.
6. Drain excess water from rack and slides; proceed to 70% alcohol for 10 dips.
7. 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.
8. 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
Mucin/Goblet cells	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. Stain in hematoxylin for a length of time to suit preference of nuclear stain intensity.
    a. Gill I recommended: Cytology – 5 minutes
    b. Gill II recommended:
        i. Frozen sections/Squash preps – 1 minute
        ii. Paraffin H&E: 3-5 minutes
        iii. IHC: 3-5 minutes
    c. Gill III recommended:
        i. Frozen sections/Squash preps – 1 minute
        ii. IHC: 3-5 minutes
4. If using a xylene substitute, closely follow the manufacturer’s recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 112.
2. Luna, Lee G. *Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts*. Gaitheresburg, MD: American Histolabs, 1992. 81-92.
3. Modifications developed by Newcomer Supply Laboratory.