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Part 1082
Revised March 2023

Eosin-Phloxine Stain Set - Technical Memo

SET INCLUDES:	**Part 1082A**
Solution A: Eosin Y Stock Stain 1%, Aqueous	1000 ml
Solution B: Phloxine B Stock Stain 1%, Aqueous	100 ml

Additionally Needed For Eosin-Phloxine Stain Set:	
Alcohol, Ethyl Denatured, 95%	Part 10842
Acetic Acid, Glacial, ACS	Part 10010

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			
Hematoxylin Stain, Harris Modified	Part 1201	or	Hematoxylin Stain, Harris	Part 12013
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 Eosin-Phloxine Stain Set solutions are aqueous based, provide a finer touch to hematoxylin and eosin stains and can be used in either manual or automated staining platforms. Differentiation of muscle, connective tissue and epithelial elements tend to be sharper and better demonstrated than with the traditional eosin y solution alone.

Hematoxylin and eosin (H&E) staining is used for screening specimens in anatomic pathology, for research, smears, touch preps and other applications. Its two primary coloring agents stain all cellular material: nuclei (blue), and cytoplasmic elements (pink-red). Popularity of this stain is due to its simplicity, ability to clearly demonstrate a variety of 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. Procedures will vary between laboratories depending upon volume of slides, automation vs manual staining, chemical hygiene and solution integrity. The longevity of eosin depends upon these factors and stain quality should be regularly screened with an H&E control slide.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 4 microns
Solutions: All solutions are manufactured by Newcomer Supply, Inc.

Standard Working Solution:

Solution A: Eosin Y Stock Stain 1%, Aqueous	100 ml
Solution B: Phloxine B Stock Stain 1%, Aqueous	10 ml
Alcohol, Ethyl Denatured, 95%	780 ml
Acetic Acid, Glacial, ACS	4 ml

Combine all solutions and mix well. Store at room temperature for up to one year.

H&E STAINING PROCEDURE WITH EOSIN-PHLOXINE:

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 Modified (1201) or Hematoxylin Stain, Harris (12013) 1-5 minutes, depending on preference of nuclear stain intensity.
3. Wash well in three changes of tap water.
4. Differentiate quickly in Acid Alcohol 1%.
    a. *Nuclei should be distinct and background very light to colorless.*
5. Rinse well 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 water; rinse in distilled water.
8. Drain excess water; proceed to 70% ethyl alcohol for 10 dips.
9. Counterstain in Eosin-Phloxine Standard 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 slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during procedure.
3. 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. 126-127.
2. Carson, Freida L., and Christa Hladik Cappellano. Histotechnology: A Self-instructional Text. 4th ed. Chicago: ASCP Press, 2015. 116-117.
3. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 143-144, 153-154.
4. Modifications developed by Newcomer Supply Laboratory.