

Eosin-Phloxine Stain Set - Technical Memo

SET INCLUDES:

Solution A: Eosin Y Stock Stain 1%, Aqueous	Part 1082A 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
Acid Alcohol 1%	Part 10011		
Lithium Carbonate, Saturated Aqueous	Part 12215	or	Scott Tap Water Substitute
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 and intended to give an extra fine touch to routine hematoxylin and eosin (H&E) 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 with better demonstration than with the traditional eosin y solution alone.

The routine 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. Eosin's particular value in the H&E stain is in its 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 eosin 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 Formalin (Part 1090)

Technique: Paraffin sections cut at 5 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. See Procedure Note #3.
5. Rinse 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 from staining rack/slides; proceed to 70% 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 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. 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.