

## Hematoxylin Stain Set, Weigert Iron - Technical Memo

### SET INCLUDES:

Solution A: Ferric Chloride, Acidified  
Solution B: Hematoxylin 1%, Alcoholic

### **Part 1409B**

250 ml  
250 ml

### **Part 1409A**

500 ml  
500 ml

### Additionally Needed:

Xylene, ACS  
Alcohol, Ethyl Denatured, 100%  
Alcohol, Ethyl Denatured, 95%

Part 1445  
Part 10841  
Part 10842

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

### APPLICATION:

Newcomer Supply Hematoxylin Stain Set, Weigert Iron is designed to be the preferred nuclear stain in conjunction with trichrome and mucin stains. Another application includes the identification of amoeba in tissue sections (see reference #2).

An iron hematoxylin solution is the optimal nuclear stain when the succeeding stains are lengthy or acidic, where the use of an aluminum-mordanted hematoxylin stain in these conditions would have a tendency to decolorize.

### METHOD:

**Fixation:** Formalin 10%, Phosphate Buffered (Part 1090)

**Technique:** Paraffin sections cut at 5 microns

**Solutions:** All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply Stain Sets are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below.

### HEMATOXYLIN, WEIGERT IRON 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. Prepare fresh Weigert Iron Hematoxylin Working Solution.
  - a. Solution A: Ferric Chloride, Acidified 20 ml
  - b. Solution B: Hematoxylin 1%, Alcoholic 20 ml
  - c. Prepare directly before use; combine and mix well.
3. Stain with fresh Weigert Iron Hematoxylin Working Solution for 10 minutes.
4. Rinse in tap water and check microscopically for good nuclear detail.
5. Wash in running tap water for 10 minutes; rinse in distilled water.
  - a. See Procedure Note #3.
6. Proceed with selected stain procedure:
  - a. Mucin stain procedure
  - b. Trichrome stain procedure
  - c. Or counterstain as desired
7. Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

### RESULTS:

Nuclei	Black
Other tissue components	Dependent on stain procedure or counterstain used

### 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. If Weigert Iron Hematoxylin is not completely washed from tissue sections, nuclear and cytoplasmic staining will be compromised.
4. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

### REFERENCES:

1. Luna, Lee G. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. 3rd ed. New York: Blakiston Division, McGraw-Hill, 1968. 228-229.
2. Preece, Ann. *A Manual for Histologic Technicians*. 3rd ed. Boston: Little, Brown, 1972. 229.
3. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 146.
4. Modifications developed by Newcomer Supply Laboratory.