



## HEMATOXYLIN PHLOXINE-SAFFRON PROCEDURE

(Revised 02/20/18)

**PRINCIPLE:** This procedure demonstrates nuclei, cytoplasm, collagen and muscle.

**SPECIMEN:** Any well fixed paraffin embedded tissue cut at 4 to 5 microns.

**QUALITY CONTROL:** American MasterTech Scientific Recommended Control Slide:  
Heart, CSH0725P; Skeletal Muscle, CSS0725P; Uterus, CSU0325P

**PROCEDURE NOTE:** If using a **Xylene Substitute**, deparaffinize slide for 10 minutes in each change with agitation; clear for 2 minutes in each change with agitation.

### **PROCEDURE:**

1. Deparaffinize slide in 2 changes of Xylene at 5 minutes each.
2. Place slide in 3 changes of Absolute Alcohol for 1 minute each with agitation.
3. Place slide in 95% Reagent Alcohol for 1 minute with agitation.
4. Rinse slide in running Tap water for 1 minute.
5. Place slide in **Harris** or **Gill's 2 Hematoxylin** for 5 minutes.
6. Rinse slide in running Tap water for 1 minute.
7. Dip slide 2 to 3 times in **Differentiating Solution**.
8. Rinse slide in running Tap water for 1 minute.
9. Place slide in **Bluing Solution** for 30 to 40 seconds.
10. Rinse slide in running Tap water for 2 to 3 minutes.
11. Place slide in **1.5% Phloxine B Solution** for 2 minutes.
12. Rinse slide in Tap water for 5 minutes.
13. Dehydrate slide through 3 changes of Absolute Alcohol.
14. Place slide in **2% Alcoholic Saffron Solution** for 5 minutes.
15. Dehydrate slide through 3 changes of Absolute Alcohol for 1 minute each.
16. Clear slide through 3 changes of Xylene for 1 minute each.
17. Coverslip using a permanent mounting media.

### **RESULTS:**

Nuclei: **BLUE**  
Cytoplasm, muscle: **RED**  
Collagen: **YELLOW**  
Red blood cells: **BRIGHT PINK TO RED**

**REFERENCE:** Sheehan DC Hrapchak BB; Theory and practice of Histotechnology; 1980, 154.

**Copyright ©2011-2018 American MasterTech Scientific. All rights reserved.**

No part of these pages may be used for any purpose other than personal use. Therefore, reproduction, modification, storage in a retrieval system or retransmission, in any form or by any means, for reasons other than personal use, is strictly prohibited without prior written permission.