

FROZEN H/E STAINING PROTOCOL

For use on Leica AutostainerXL (Last Updated 3/24/23)

OBJECTIVE: To identify nuclei verses cytoplasm and other tissue elements

RECOMMENDED CONTROL TISSUE: Appendix, small bowel or any well-fixed tissue routinely stained in lab.

ID of Control Used: _____ **Type of Tissue:** _____

Reagent	Vendor	Catalog #	Was this a new Bottle (Y/N)? Yes, check lot log.
Harris Hematoxylin	Epredia	72711	
Glacial Acetic Acid	Fisher	A35-500	
Ammonium Hydroxide	Fisher	A669-500	
Eosin Phloxine 515	Surgipath	3801606	

SPECIAL INSTRUCTIONS: Hematoxylin – Filter Daily (if new or reused). Milli-Q H₂O, Bluing and Acid Alcohol – change daily. Hematoxylin and Eosin – Change Biweekly or after 400 slides. Dump all other reagents after 200 slides or as needed. Rotate/change as needed.

REAGENT PREPARATION:

*Acid Alcohol – Add 3ml of Glacial Acetic Acid to 3L 70% Ethanol.

**Ammonia water – Add 1ml Ammonia Hydroxide to 3.8L DI H₂O.

PROTOCOL:

1. Prepare 4µm frozen sections on glass slides
2. Air dry slides for 15 min.
3. Fix slides in 95% ETOH for 10 mins.
4. Rinse slides 3X in DIH₂O
5. Run Program 2 on Leica Autostainer XL. Place rack into Load drawer and press ‘Load’. Program is as follows:
 - a. Station 5 95% ETOH 1 min.
 - b. Station 6 Milli Q H₂O 50 secs.
 - c. Station 8 Harris Hematoxylin 2 min
 - d. DI WASH 5 1:45 mins.
 - e. Station 9 0.1% Acid Alcohol* 5 sec
 - f. WASH 4 30 secs.
 - g. Station 10 Ammonia Water** 45 secs.
 - h. WASH 3 30 secs.
 - i. Station 11 70% ETOH 10 secs.
 - j. Station 12 Eosin 1 min.
 - k. Station 13 95% ETOH 10 secs.
 - l. Station 14 100% ETOH 10 secs.
 - m. Station 15 100% ETOH 10 secs.
 - n. Station 16 Xylene 25 secs.
 - o. Station 17 Xylene 25 secs.
 - p. Station 18 Xylene 25 secs.
 - q. EXIT Xylene EXIT.
6. Machine beeps when cycle is complete; Press Exit and remove rack.
7. Coverslip

EXPECTED RESULTS: Nuclei – blue, Cytoplasm and other tissue – various shades of pink, Erythrocytes, and eosinophilic granules – bright pink to red.

CASE IDs/SLIDE NUMBERS: