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: ____________________

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Vendor</th>
<th>Catalog #</th>
<th>Was this a new Bottle (Y/N)?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harris Hematoxylin</td>
<td>Epredia</td>
<td>72711</td>
<td>Y</td>
</tr>
<tr>
<td>Glacial Acetic Acid</td>
<td>Fisher</td>
<td>A35-500</td>
<td></td>
</tr>
<tr>
<td>Ammonium Hydroxide</td>
<td>Fisher</td>
<td>A669-500</td>
<td></td>
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<tr>
<td>Eosin Phloxine 515</td>
<td>Surgipath</td>
<td>3801606</td>
<td></td>
</tr>
</tbody>
</table>

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: