Tissue Staining with Hematoxylin and Eosin (H&E)

Health and Safety:

Wear nitrile gloves, laboratory coat and safety glasses. The process prefers to be done with fume hood ventilation.

Equipment and Materials:

Reagents:

Water from Milli-Q System water (18 mega-ohm, TOC < 5 ppb), and Distilled/deionized water; Ethanol: (EtOH); Phloxine B; Xylene: Histological grade (e.g. Acros Organics Cat. No 1330-20-7)

Eosin Solution:

Eosin Y solution, Intensified, histology grade (e.g. Fisher Cat. No 314-630)

Hematoxylin solution:

- Hematoxylin: (e.g. Sigma-Aldrich Cat. No H-3136)
- Aluminum potassium sulfate 12-hydrate
- Distilled/deionized water
- Glycerol (e.g. Acros Organics)
- Sodium iodate

Other supplies:

Slide Preparation Mounting Medium: Xylene-based (e.g. Cytoseal XYL, Richard-Allan Scientific Cat. No 8312-4)

Microscope cover slides (e.g. Fisher Cat. No 12-548-C 25x25 mm and Fisher Cat. No 12-548-5M 24x50 mm)

Filter paper (e.g., Fisher)

Procedure:

Preparation of the staining solutions

Ethanol solutions: make 500 mL 95% ethanol and 500 mL 70% ethanol solution with distilled/dionized water

Hematoxylin solution (Store in dark, the solution is good for approximately six months.)

1. Dissolve 1 g hematoxylin in 100 mL glycerol
2. Dissolve 45.93 g aluminum potassium sulphate dodecahydrate in 354 mL distilled water.
3. Dissolve sodium iodate in 25 mL distilled water.
4. Add the aluminum potassium sulphate solution to the hematoxylin solution slowly, while mixing well.
5. Add the 0.1 g sodium iodate solution, mix well.
6. Gravity-filter before use.

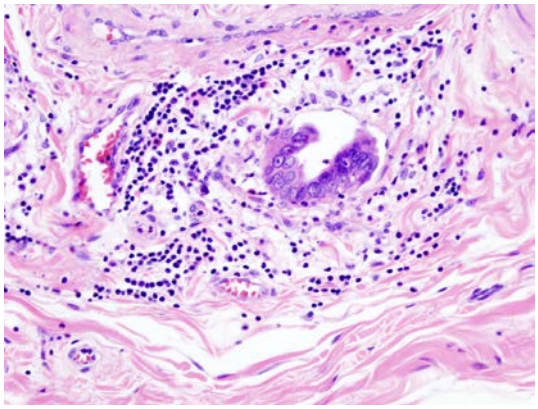
Eosin solution:

Dissolve 1 g phloxine B in 100 mL distilled water to make 1% phloxine B stock solution. Add 0.57 mL of phloxine B stock solution to 50 mL Eosin Y solution. Mix well. Solution is good for approximately six months.

Tissue staining:

1. 30 seconds in 95% ethanol
2. 30 seconds in 70% ethanol
3. 30 seconds in Milli-Q water
4. 2 minutes in hematoxylin solution (deep purple; stains nuclear contents)
5. 20 seconds in Milli-Q water
6. 30 seconds in 70% ethanol
7. 30 seconds in 95% ethanol
8. 1 minute in eosin solution (pink; stains cytoplasm)
9. 30 seconds in 95% ethanol
10. 30 seconds in 100% ethanol
11. 2-2.5 minutes in Xylene
12. Cover-slips: (If the slide is used for laser capture micro-dissection, let it air dry for 5 minutes and store in a desiccator without cover-slipping.) To use cover-slips, dry the back of the microscope slide and around the tissue section with a Kimwipe to get rid of excess xylene. Put 1-3 drops of Cytoseal on the tissue section and then cover with a cover slide. Make sure no air bubbles are left under the slide. Let dry until Cytoseal has hardened.

Expected Outcome:



The nuclear contents will appear blue-purple, red blood cells red and other cellular and extracellular material pink.

For best results, the tissue sections should be stored in cold (-20 °C) or in 10% buffered formalin until staining (if stored in formalin, leave no more than a few hours, if frozen at -20°C, it can be more than a year). Putting them in a desiccator causes the cells to dry out, resulting in compromised cellular morphology. To perform an H&E stain, follow the procedure below to re-hydrate, dehydrate and stain the tissue sections. Same solvents can be used for multiple tissue sections but should be changed daily or more often if they become contaminated (strongly colored by the stains).

References:

1. Laboratory methods in histotechnology / edited by: Edna B. Prophet ... [et al.]; prepared by the Armed Forces Institute of Pathology
2. Haematoxylin and Eosin staining: Oversights and insights – Gary W. Gill, DAKO Special stains, second edition.