Hematoxylin & Eosin (H&E) Stain

For staining nuclei (hematoxylin) and cytoplasm (eosin).

It is useful to first stain with H&E to identify the nature of the tissue section before proceeding with decisions on further stains and/or immunostains. An H&E stain – in fact, any dye used to stain tissue sections – will generate fluorescent signals. Thus, dye-stained sections are great to have on-hand when trying to focus on specimens that have been labelled for viewing using epifluorescence light.

After completing an **immunostain** on tissue,

- **Do not use eosin** as it will confound the results.
- If you completed an immunostain and the **colored substrate is aqueous**, remember that **hematoxylin is also aqueous** (Mayer's not Gill's).

Procedure (Frozen Sections)

1. Fix frozen sections in 10% buffered formalin (Fisher Scientific, cat. #SF93-4) for *20 min* and then wash in water
2. Stain nuclei by immersing in Gill II Hematoxylin (Surgipath, cat. #01522) for *3 min* and then wash in water
3. Immerse in bluing agent (Fisher Scientific, cat. #CS410-4) for *30 sec*, then wash in water
4. Immerse in 95% ethanol briefly
5. Stain cytoplasm by immersing in Eosin (Surgipath, cat. #01602) for *3 min* and dip once quickly in water
6. Dehydrate by immersing in 1 change of 95% ethanol for *1 min*, and then in 3 changes of 100% ethanol (Fisher Scientific, cat. #A962p) for *10 dips* ***each***
7. Immerse in 3 changes of Citrosol (Fisher Scientific, cat. #22-143-975) for *2 min* ***each***
8. Mount slides with Cytoseal 60 (VWR, cat. #48212-154) using glass coverslips (Surgipath, cat. #00145)

H&E Stain on Frozen Sections for Laser Capture Microscopy (Simone *et al.* 2000)

1. Thaw frozen sections (on plain untreated glass slides) one at a time to decrease degradation
2. Fix with 70% ethanol for *10 sec*, then wash in deionized water
3. Immerse in **fresh** Mayer's hematoxylin for *30 sec*, then wash in water
4. Immerse in bluing reagent for *30 sec*, then wash in 70% ethanol
5. Immerse in eosin for 90 sec
6. Dehydrate sections with ***two*** *10-sec* washes in 90% ethanol followed by ***two*** *10-sec* washes in 100% ethanol
7. Place in xylene for *30 sec*

Simone NL, Remaley AT, Charboneau L, Petricoin III EF, Glickman JW, Emmert-Buck MR, Fleisher TA & Liotta LA. 2000. Sensitive Immunoassay of Tissue Cell Proteins Procured by Laser Capture Microdissection. Am J Pathol. 156(2):445-452. PMID: 10666374. PMCID: PMC1850045. doi: 10.1016/S0002-9440(10)64749-9

Procedure (Paraffin Sections)

1. Deparaffinize by immersing successively in 2 changes of xylene (Fisher Scientific, cat. #X3P) for *10 min* ***each***
2. Rehydrate by immersing in decreasing concentrations of ethanol (Fisher Scientific, cat. #A962p) as follows:
    a. 100% ethanol for *5 min*
    b. 100% ethanol for *5 min*
    c. 95% ethanol for *5 min*
    d. 95% ethanol for *5 min*
3. Immerse in water for *5 min*
4. Stain nuclei by immersing in Gill II Hematoxylin (Surgipath, cat. #01522) for *3 min* and then wash in water
5. Immerse in bluing agent (Fisher Scientific, cat. #CS410-4) for *30 sec*, then wash in water
6. Immerse in 95% ethanol briefly
7. Stain cytoplasm by immersing in Eosin (Surgipath, cat. #01602) for *3 min* and dip once quickly in water
8. Dehydrate by immersing in 1 change of 95% ethanol for *1 min*, and then in 3 changes of 100% ethanol (Fisher Scientific, cat. #A962p) for *10 dips* ***each***
9. Immerse in 3 changes of Citrosol (Fisher Scientific, cat. #22-143-975) for *2 min* ***each***
10. Mount slides with Cytoseal 60 (VWR, cat. #48212-154) using glass coverslips (Surgipath, cat. #00145)