Overview

The following is protocol for performing an H&E stain. “H&E is the combination of two histological stains, [hematoxylin](https://en.wikipedia.org/wiki/Haematoxylin) and [eosin](https://en.wikipedia.org/wiki/Eosin), the process stains cell [nuclei](https://en.wikipedia.org/wiki/Cell_nucleus) blue, and [extracellular matrix](https://en.wikipedia.org/wiki/Extracellular_matrix) and [cytoplasm](https://en.wikipedia.org/wiki/Cytoplasm) pink, with other structures taking on different shades, hues, and combinations of these colors.[[5]](https://en.wikipedia.org/wiki/H%26E_stain#cite_note-Chan,_2014-5)[[6]](https://en.wikipedia.org/wiki/H%26E_stain#cite_note-Bancroft_and_Stevens,_1982-6) The stain shows the general layout and distribution of cells and provides a general overview of a tissue sample's structure” (taken from [Wikipedia](https://en.wikipedia.org/wiki/H%26E_stain)). It can be used on a variety of tissue types for several reasons.

Additional resources

[Wikipedia on H&E Stain](https://en.wikipedia.org/wiki/H%26E_stain)

[H&E Staining Overview: A Guide To Best Practices](https://www.leicabiosystems.com/us/knowledge-pathway/he-staining-overview-a-guide-to-best-practices/)

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| **Troubleshooting Guides:** |
| [H&E Troubleshooting Guide (Table)](https://documents.cap.org/documents/h-and-e-troubleshooting-guide.pdf) |
| [The Science and Application of H&E Staining](https://boneandcancer.org/__static/e9868a4f6b4df8f1b185756e61c6ce30/f-8-b-the-science-and-application-of-h-and-e-staining-by-skip-brown-nwu(2).pdf?dl=1) |
| [H&E Basics: Troubleshooting](https://www.leicabiosystems.com/us/knowledge-pathway/he-basics-part-4-troubleshooting-he/) |
| [Troubleshooting H&E Stains](https://www.nsh.org/blogs/natalie-paskoski/2020/05/15/troubleshooting-he-stains) |

Main content

**Materials**

* Hematoxylin (Abcam, SKU# ab220365)
* Acidic Ethanol (used for differentiating Hematoxylin in some H&E protocols, but not needed for this protocol)
  + 401mL Stock
    - 1mL 12M HCl
    - 400mL 70% Ethanol (= 280mL 100% Ethanol + 120mL diH2O)
  + 802mL Stock
    - 2mL 12M HCl
    - 800mL 70% Ethanol (= 560mL 100% Ethanol + 240mL diH2O)
* Bluing Reagent (Abcam, SKU# 67069)
  + 1 g Sodium Bicarbonate
  + 1 L diH2O
* 1.0% Eosin Y Stock
  + 1.0g Eosin Y
  + 100mL diH2O
* 1.0% Phloxine B Stock
  + 1.0 g Phloxine B
  + 100mL diH2O
* Alcoholic Eosin Y/Phloxine B Working Solution
  + 25mL 1.0% Eosin Y Stock
  + 2.5mL 1.0% Phloxine B Stock
  + 195mL 95% Ethanol ( = 185mL 100% Ethanol + 10mL diH2O)
  + 2mL Glacial Acetic Acid (Concentration = 0.89%)
* 2x 95% Ethanol (for rinsing slides)
* 2x 100% Ethanol (for rinsing slides)
* Tap Water (for hydration and rinsing)

**Assay Procedure**

1. Apply Hydrophobic Barrier Pen around tissue sections
2. Fixate slides in **10% Neutral Buffered Formalin** for 30 minutes
   1. Hydrate in tap water for 1 minute.
3. Drop **Hematoxylin** onto tissue sections; let sit for 25 seconds.
   1. Rinse in tap water until the water runs clear or until the glass around the tissue is no longer purple (minimum 2 minutes).
4. Drop **Bluing Reagent** onto sections; let sit for 1 minute
   1. Rinse in tap water for 1 minute.
   2. Dip in 95% Ethanol for 30 seconds.
5. Dip in **Eosin Y/Phloxine B Working Solution** for 30 seconds.
   1. Rinse in 70% Ethanol for 15 seconds to rinse off excess Eosin.
   2. Dip in fresh 70% Ethanol for 30 seconds to differentiate Eosin.
   3. Dip in 95% Ethanol for ~20-30 seconds.
   4. Dip in fresh 95% Ethanol for ~20-30 seconds.
   5. Dip in 100% Ethanol for ~20-30 seconds.
   6. Dip in fresh 100% Ethanol for ~20-30 seconds.
6. Air dry slides before clearing in Xylene.
7. Clear in **Xylene** for at least 15 mins, until the hydrophobic barrier pen border is washed off the slide.
8. Air dry slides again.
9. Dip in **fresh Xylene** again to wet slides before applying cover slip.
   1. Apply DPX while slide is still wet with Xylene, then place cover slip.

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| **H&E Staining Protocol**  Section thickness = 10μm | |
| *Apply Hydrophobic Barrier Pen around tissue sections* | |
| **10% Neutral Buffered Formalin** | **30 mins** |
| Hydrate in tap water | 1 min |
| **Hematoxylin** | **25 secs** |
| Rinse in tap water | ~2 mins |
| **Bluing Reagent** | **1 min** |
| Rinse in tap water | 1 min |
| 95% Ethanol | 30 secs |
| **Eosin Y/Phloxine B Working Solution** | **30 secs** |
| 70% Ethanol | 15 secs |
| Fresh 70% Ethanol | 30 secs |
| 95% Ethanol | ~20-30 secs |
| Fresh 95% Ethanol | ~20-30 secs |
| 100% Ethanol | ~20-30 secs |
| Fresh 100% Ethanol | ~20-30 secs |
| *Air dry slides before clearing in Xylene* | |
| **Xylene** | **15+ mins** |
| *Air dry slides again* | |
| Dip in **fresh** **Xylene** before applying mounting medium | |
| Add DPX mounting medium while Xylene is freshly wet, then place cover slip | |