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) |
| [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
  + 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
* Eosin Y Stock
  + 400mL of 80% Ethanol (= 320mL 100% Ethanol + 80mL diH2O)
  + 0.8g Eosin Y
  + 0.05g Phloxine B
  + 1.4mL Glacial Acetic Acid
* 95% Ethanol (for rinse)
* 100% Ethanol (for rinse)
* Tap Water (for hydration and rinse)

**Assay Procedure**

1. Fixate slides in 10% Neutral Buffered Formalin for 30 minutes
2. Hydrate in Tap Water for 1 minute
3. Dip in Hematoxylin for 2 minutes.
4. Rinse in tap water until the water runs clear.
5. Dip for 30 seconds in **Acidified Ethanol**
6. Rinse in tap water
7. Dip in **Bluing Reagent** for 1 min
8. Rinse in tap water
9. Dip for 30 seconds in 95% ethanol.
10. Dip in **Eosin** for 1 minute.
11. Dip for 30 seconds in 70% ethanol to rinse of Eosin
12. Dip for 1 minute in:
    1. 70% ethanol
    2. 95% ethanol
    3. 100% ethanol
13. Air dry slides before clearing in Xylene
14. Clear in Xylene
15. Air dry slides
16. Dip in Xylene again to wet slides before applying cover slip
    1. Apply DPX while slide is still wet with Xylene
    2. Apply cover slip over DPX