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
* Eosin Y Working Solution
  + Dilute stock solution 1:1 with 70% Ethanol
  + Add 1mL Glacial Acetic Acid per 50mL of Eosin Y Working Solution
* 95% Ethanol (for rinse)
* 100% Ethanol (for rinse)
* Tap Water (for hydration and rinse)

**Assay Procedure**

1. Apply Hydrophobic Barrier Pen around tissue sections
2. Fixate slides in **10% Neutral Buffered Formalin** for 30 minutes
3. Hydrate in tap water for 1 minute
4. Dip in **Hematoxylin** for 2 minutes.
5. Rinse in tap water until the water runs clear.
6. Dip for 30 seconds in **Acidified Ethanol**
7. Rinse in tap water
8. Dip in **Bluing Reagent** for 1 min
9. Rinse in tap water
10. Dip for 30 seconds in 95% ethanol.
11. Dip in **Eosin** for 1 minute.
12. Dip for 30 seconds in 70% ethanol to rinse of Eosin
13. Dip for 2 minutes in fresh 70% ethanol
14. Dip for 1 minute in:
    1. 95% ethanol
    2. 100% ethanol
15. Air dry slides before clearing in Xylene
16. Clear in Xylene for 10-15 mins, until the hydrophobic barrier pen border is washed away
17. Air dry slides again
18. Dip in 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** | 2 mins |
| Rinse in tap water | 1 min |
| **Acidified Ethanol** | 30 secs |
| Rinse in tap water | 1 min |
| **Bluing Reagent** | 1 min |
| Rinse in tap water | 1 min |
| 95% Ethanol | 30 secs |
| **Eosin Y Working Solution** | 2 mins |
| 70% Ethanol | 30 secs |
| 70% Ethanol | 2 mins |
| 95% Ethanol | 1 min |
| 100% Ethanol | 1 min |
| *Air dry slides before clearing in Xylene* |  |
| **Xylene** | 10-15 mins |
| *Air dry slides again* |  |
| Final dip in **Xylene** before applying mounting medium |  |
| Add DPX mounting medium while Xylene is freshly wet, then apply cover glass | |