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)

Need more help?

Check the resources, and then see Ken

Main content

**Materials**

* Methanol
* Ethanol
* Hydrochloric Acid
* ~~Ammonia Water~~
  + ~~5 Drops of NH~~~~4~~~~OH~~
  + ~~100mL of ddH~~~~2~~~~O~~
  + Acidic Ethanol:
    - 1mL 12M HCl
    - 400mL 70% Ethanol
      * 280mL 100% Ethanol
      * 120mL diH2O
  + Bluing Reagent (Abcam, SKU# 67069)
    - 1 gram Sodium Bicarbonate
    - 1 L diH2O
* Hemotoxylin (Abcam, SKU# ab220365)
* Eosin
  + 400mL of 80% Ethanol
  + 0.8g Eosin
  + 0.05g Phloxine B
  + 1.4mL Glacial Acetic Acid

**Materials**

1.      Put slides into 100% methanol for fixation, for 5 minutes

2.      Dip for 30 seconds each in:

a.       100% Ethanol

b.      100% Ethanol

c.       95% Ethanol

d.      95% Ethanol

e.       70% Ethanol

f.       Tap Water

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 2 minutes.

11.  Dip for 30 seconds in same 95% ethanol from step 9 to rinse of Eosin

12.  Dip for 1 minute in:

a.       95% ethanol

b.      100% ethanol

c.       Xylene

13.  Allow slides to air dry.