

VERSION 2  
SEP 19, 2023

OPEN ACCESS



**DOI:**  
[dx.doi.org/10.17504/protocols.io.36wgq3n2ylk5/v2](https://dx.doi.org/10.17504/protocols.io.36wgq3n2ylk5/v2)

**Protocol Citation:** sbreslin 2023. Buck Institute Morphology H & E staining protocol. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.36wgq3n2ylk5/v2> Version created by sbreslin

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**Protocol status:** Working  
We use this protocol and it's working

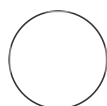
**Created:** Sep 19, 2023

## Buck Institute Morphology H & E staining protocol V.2

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<sup>1</sup>Buck Institute for Research on Aging

sbreslin: Morphology Core standard protocol



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### ABSTRACT

Histological Stain for FFPE slides

**RESULTS:** Nuclei-blue; Cytoplasm, red blood cells and connective tissue-shades of pink

### MATERIALS

1. Modified Mayes's Hematoxylin HXMMHPT StatLab Medical Products
2. 10% Acidic Acid
3. Scott's Tap Water (1L + 3.5g Sodium Bicarb + 20g Magnesium Sulfate pH 8.0) - Bluing Agent
4. Eosin Y Stain STE0150 StatLab Medical Products
5. reagents in 250 ml Coplin Jar

### SAFETY WARNINGS

- Xylene (C<sub>8</sub>H<sub>10</sub>)CAS No. 1330-20-7 is a colorless, flammable liquid with a sweet odor. Exposure to xylene can irritate the eyes, nose, skin, and throat. Xylene can also cause headaches, dizziness, confusion, loss of muscle coordination, and in high doses, death. Workers may be harmed from exposure to xylene.  
from <https://www.cdc.gov/niosh/topics/xylene/>  
Permunt Mounting Media is toluene-based synthetic resin mounting medium. Use caution around uncured media.  
<https://www.cdc.gov/niosh/topics/toluene/>

### BEFORE START INSTRUCTIONS

Slides can be placed in 60 C oven prior to deparaffinization.

## Hematoxylin and Eosin Staining

- 1** Deparaffinization and rehydration: 2 x xylene 7 min, 100% ethanol 4 min, 95% 4 min, 80% 4 min, 70% 4 min, bring to water.
- 2** Hematoxylin staining for 30 seconds - 4 min. using filtered dye (usually 2 mins) - Modified Mayes's Hematoxylin HXMMHPT StatLab Medical Products
- 3** Rinse in ddH2O x2
- 4** 10% acetic acid rinse (acid into water) – 1 min
- 5** rinse in ddH2O x2
- 6** TBS (1x) rinse or Scott's Tap Water (1L + 3.5g Sodium Bicarb + 20g Magnesium Sulfate pH 8.0) - 2 mins
- 7** Rinse in ddH2O

- 8** Rinse in 70% ETOH - 1 min
- 9** Stain Eosin for 0.5 to 2 minutes (keep an eye on intensity of pink) – Eosin Y Stain STE0150 StatLab Medical Products
- 10** Rinse in 90% EtOH
- 11** Rinse in 2 x 95% EtOH, 2 x 100% EtOH, xylene
- 12** Coverslip with Permount Mounting Medium EMS #17986-01