

Dec 11, 2019

UF / HuBMAP - H&E Staining Process V.2

Franchesca Farris¹, Marda Jorgensen¹, Leigh Propper¹¹University of Florida

1

Works for me

dx.doi.org/10.17504/protocols.io.9qbh5sn

Human BioMolecular Atlas Program (HuBMAP) Method Development Community

ABSTRACT

This protocol will detail our method of utilizing the H&E stain as a nonspecific entity to achieve optimal results in our staining process for our research samples.

Hematoxylin and Eosin stains are used in many areas of the laboratory, including frozen sections, fine needle aspirates, and paraffin fixed embedded tissues. There are multiple factors involved in determining what makes a well stained slide, and one of the primary factors involved in the final product is pathologist preference. Other important factors also play important roles in your final stain, such as reagents and their quality, consistency, initial microtomy quality and selecting a sound procedure.

Hematoxylin is used to illustrate nuclear detail in cells. The resulting intensity and shade of the dye in the cells is primarily reliant on the length of time the sample spends in hematoxylin. The cytoplasmic component of the section is brought out by Eosin, which is the most commonly used counterstain in histology. It's varying shades of pink and red provide an exceptional contrast between the nuclear and cytoplasmic areas of interest.

GUIDELINES

- Managers and supervisors - are responsible for making sure that technicians are properly trained and equipment and facility are maintained in good working order.
- Laboratory personnel - are responsible for reading and understanding this SOP and related documents and to perform these tasks in accordance with the SOPs.

MATERIALS

NAME ▾	CATALOG # ▾	VENDOR ▾
Water		
Xylene	X3P-1GAL	Fisher Scientific
Richard-Allan Scientific™ High-Quality Dehydrants, Reagent Alcohol	9111	Thermo Fisher
Eosin-Y OPTIK	RS4359-A	
Hematoxylin OPTIK DK	RS4576-A	
Bluing Reagent OPTIK	RS-4363-B	
Aqueous Clarifier	RS4361-B	

SAFETY WARNINGS

- Use universal safety precautions when handling human samples and personal protective equipment (e.g., face mask with shield, gloves, lab coat or apron).
- Use chemical and physical safety precautions when working with paraformaldehyde and sharps, respectively.
- Perform under a hood if available.
- Gloves should be worn when performing staining process

BEFORE STARTING

- Be sure to run a control slide prior to patient/research slides, or run at the same time.
- Confirm you have appropriate quantities of the reagents required.
- Verify that all slides have been dried in an oven prior to staining.

- 1 Ensure your staining line or staining set up has sufficient reagents and is correctly set up for use.

5m



Example of a H&E stain line.

- 2 Place slides into a xylene compatible slide rack.

30s

- 3 Immerse slide holder in the first xylene staining pot for 5 minutes.

5m

- 4 Immerse slide holder in the second xylene staining pot for 5 minutes.

5m

- 5 Remove slide holder from xylene and transfer to the first staining pot of 100% EtOH for 5 minutes.

5m



Be sure to blot excess xylene before going into ethanol.

- 6 Remove slide rack from first EtOH dish, and transfer to the second EtOH pot for an additional five minutes.

5m

- 7 Transfer to 95% EtOH for 3 minutes.

3m

- 8 Transfer to 70% EtOH for 3 minutes.

3m

- 9 Transfer to 50% EtOH for 3 minutes.

3m

10 Transfer to water for 2 minutes. 2m



While sections are in water, skim surface of hematoxylin with a Kimwipe to remove oxidized particles.

11 Transfer to the second change of water for an additional 2 minutes. 2m



Blot excess water from slide holder before going into hematoxylin.

12 Transfer slides to Hematoxylin for 1 minute and 15 seconds. 1m 15s

13 Rinse in de-ionized water, and then transfer slides to tap water for 30 seconds. 30s

14 After rinsing in tap water, place slide rack back into the first dish of water for 1 minute. 1m

15 Transfer to second water staining dish for 1 minute. 1m

16 Transfer to Aqueous Clarifier for 45 seconds. 45s

17 Do a quick rinse in de-ionized water and then transfer to tap water for 1 minute. 1m

18 Transfer to bluing reagent for 1 minute. 1m

19 Place in water for 5 minutes. 5m

20 Transfer to 80% EtOH for 1 minute. 1m

21 Transfer to Eosin-Y for 1 minute. 1m

22 Transfer to 100% EtOH for 2 minutes. 2m

23 Transfer to the second 100% EtOH for 5 minutes. 5m




Be sure to blot excess ethanol before going into xylene.

24 Transfer to xylene for 5 minutes. 5m

25 Transfer to next xylene pot for 5 minutes. 5m

26 After the second xylene change, use a xylene compatible mountant to affix a coverslip to the slide. 3m

 This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited