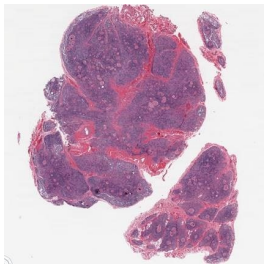
Dec 12, 2019 Version 2

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



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Franchesca Farris[1], Marda Jorgensen[1], Leigh Propper[1]

[1]University of Florida

Human BioMolecular Atlas Program (HuBMAP) Method Development Community
Tech. support email: **Jeff.spraggins@vanderbilt.edu**

Franchesca Farris

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Protocol status: Working
We use this protocol in our group and it is working.

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Keywords: h&e, hematoxylin, eosin, uf, ufl, hubmap, university of florida, npod, eosin stain, staining process, nuclear detail in cell, histology, counterstain in histology, final stain, cytoplasmic area, cytoplasmic areas of interest, cytoplasmic component, cytoplasmic component of the section, initial microtomy quality, dye, nuclear detail, cell, embedded tissue, shade of the dye, many areas of the laboratory, stained slide

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

MATERIALS

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

Troubleshooting

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

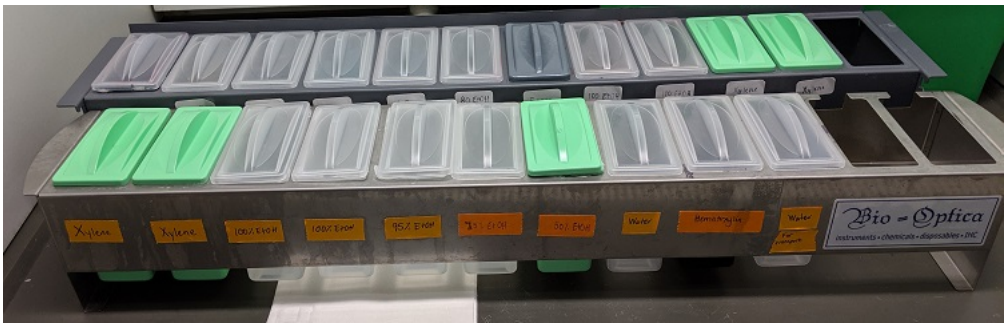
Before start

- 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

Note

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

Note

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

Note

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

Note

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