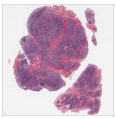

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UF H&E Staining

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1 Works for me dx.doi.org/10.17504/protocols.io.beamjac6

Human BioMolecular Atlas Program (HuBMAP) Method Development Community

ABSTRACT

Hematoxylin and Eosin stains are the prefered method for histopathologic assessment of tissue sections.

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 identified 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	
Shandon Mountant	1900231	Fisher Scientific

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).

- Xylenes and ethanol are flammable. Avoid open flames and perform procedure in a fume hood .

-Gloves should be worn when performing staining process

BEFORE STARTING

Confirm an adequate level of the reagent in each staining line station.

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Verify that all slides are completely dry. Slides can be dried in an 55C oven for one hour prior to use.

1 Prepare staining line with reagents in order of use, based on steps three through 25.

Example of a H&E stain line.

2 Place slides into a xylene compatible slide rack.

3 Immerse slide holder in the first xylene staining station for 5 minutes. 5m

4 Immerse slide holder in the second xylene staining station for 5 minutes. 5m

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

QUickly blot excess xylene from rack before transfering into ethanol.

6 Transfer slide rack into the second EtOH station 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

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

Quickly blot excess water from slide holder before going into hematoxylin.

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

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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 de-ionized 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 station 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 a fresh xylene station for 5 minutes. 5m

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

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