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🌐 UF H&E Staining

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We use this protocol and it's working

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ABSTRACT

Hematoxylin and Eosin stains are the preferred 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. Its 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

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☒ Water **Contributed by users**

☒ Xylene **Fisher Scientific Catalog #X3P-1GAL**

☒ Richard-Allan Scientific™ High-Quality Dehydrants, Reagent Alcohol **Thermo Fisher Catalog #9111**

☒ Eosin-Y OPTIK **Contributed by users Catalog #RS4359-A**

☒ Hematoxylin OPTIK DK **Contributed by users Catalog #RS4576-A**

☒ Bluing Reagent OPTIK **Contributed by users Catalog #RS-4363-B**

☒ Aqueous Clarifier **Contributed by users Catalog # RS4361-B**

☒ Shandon Mountant **Fisher Scientific Catalog #1900231**

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 START INSTRUCTIONS

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

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

Note

QUickly blot excess xylene from rack before transferring 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
- Note**

Quickly 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 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

Note

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.