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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. Depth of coloration is not only related to the amount of DNA in the nuclei but also to the length of time the sample spends in hematoxylin. Eosin is the most commonly used counterstain that distinguishes between the cytoplasm and nuclei of cells. It is typically pink, with different shades of pink for different types of connective tissue fibers. (Note: Bluing reagents, such as Scott's Tap Water, are used to change the hematoxylin from red to the traditional blue color we expect. These slightly basic solutions chemically alter the dye to produce this color change.)

GUIDELINES

- The water steps act as good pause points if a short break (~15 minutes) is needed.
- If the final water (before the 95% Ethanol) has a slight purple color to it, it can be changed and do a short dip into the clean water.
- Check the Hematoxylin staining by looking at it quickly under a light microscope after the final water step making sure the section does not wash out. If the Hematoxylin is not dark enough, it can be placed back into the Hematoxylin for another 30 seconds, followed by the subsequent water steps. (*Note: do not leave in hematoxylin for too long*)
- Before final Xylene baths + mounting, the quality of the staining can be checked under the light microscope. This should be very brief to prevent the slides from drying out. If the Eosin is not dark enough the slides can be placed back into the first 95% Ethanol before the Eosin/Phloxine step and processed through once more.

MATERIALS

·Coverglass
Epredia™ Shandon-Mount™ **Fisher Scientific Catalog #1900333**
·Cotton Tipped Applicators
·Paper Towel
·Slide Folders
·Kimwipes
·Tissue-Tek Slide Staining Set
Eosin Y Solution, Alcoholic, with Phloxine **Merck MilliporeSigma (Sigma-Aldrich) Catalog #HT110316**
Mayer's Hematoxylin Solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #MHS16-500ML**
Epredia™ Xylene **Fisher Scientific Catalog #22-050-283**
Sodium hydroxide solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #415413**
Epredia™ Dehydrant Alcohol **Fisher Scientific Catalog #22-050-106**
·Water
·Whatman filter paper
·1 funnel

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. All Xylene procedures need to be performed in a fume hood.
- Gloves should be worn when performing staining process.

BEFORE START INSTRUCTIONS

- Make sure that the level of the reagent in each staining line station is the required one.
- Verify that all slides are completely dry before the staining starts.

1. Prepare the bluing solution by adding 1 mL of 28% Sodium Hydroxide to 400 mL of water.
2. The Hematoxylin should be gravity filtered through the Whatman filter paper before use.
3. The Eosin is in a ready-to-use state.

Staining Procedure

1 Prepare staining line with reagents in the staining train in the order of use.

2 Place slides into a xylene compatible slide rack and start the deparaffination and rehydration steps.

	A	B
	Xylene (I)	4 min
	Xylene (II)	4 min
	Xylene (III)	4 min (1*)
	100% Ethanol (I)	1 min
	100% Ethanol (II)	1 min
	95% Ethanol	1 min
	70% Ethanol	1 min
	Water (I)	5 min
	Water (II)	5 min
	Water (III)	5 min (2*)

Note 1: Blot excess xylene from rack before transferring into ethanol (don't let the slide dry).*
Note 2: Blot excess water from rack before transferring into hematoxylin (don't let the slide dry).*

3 Place the slides in Hematoxylin for 1 minute. Adjust timing by 30 second intervals to lighten or darken the stain.

4 Wash slides off with water until the purple color is no longer coming off into the water. Leave them in the final water for 5 minutes.

5 Transfer to bluing reagent for a brief dip.

6 Wash the slides with 3 changes of water and leave in the final water for 5 minutes.

7 Transfer to 95% ethanol for 1 minute.

8 Transfer to Eosin-Y for 1 minute.

9 Transfer to 70% ethanol for 5 minute.

10 Follow the dehydration steps:

	A	B
	95% Ethanol	5 min
	100% Ethanol (I)	5 min
	100% Ethanol (II)	5 min (*)
	Xylene (I)	5 min
	Xylene (II)	5 min

*Note *: Blot excess ethanol from rack before transferring into xylene (don't let the slide dry).*

11 Coverslip the slides by removing them from the final xylene (1 at a time) and dabbing the edges with paper towel to absorb some excess Xylene. Do not allow the section to dry out.
Place 1-2 drops of mounting media on the tissue section and carefully place the coverslip starting on at an angle and laying over the tissue to avoid air bubbles.
Carefully use the cotton tipped applicator to push out any excess mounting media and air bubbles.

12 Place the slides, tissue side up, in a slide folder to dry overnight.

Note: A Kimwipe tissue can be used to wipe off the applied Xylene and cleaning any residual debris from the slide.

Imaging

13 Slides will then be scanned by the Center for Biologic Imaging (CBI) using a VS200 slide scanner. Images can be acquired with brightfield scans at (10X, 20X).