Hematoxylin and Eosin Stain of FFPE Tumor Tissue

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1 Works for me This protocol is published without a DOI.

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ABSTRACT

This protocol describes the steps for doing a manual Hematoxylin and Eosin (H&E) Stain of formalin fixed tissue. This stain is used to distinguish tumor tissue, background normal and stromal tissue, as well as tumor infiltrating immune cells. All pathology reviews of tumor tissue received from study participants is performed by examining the H&E stained tissue.

PROTOCOL CITATION

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GUIDELINES

This protocol is for FFPE tissues only.
Staining with Hematoxylin and Eosin can vary over time and staining length may need to be empirically determined. If staining becomes too faint or uneven, new reagents will need to be substituted.
It is important to use tap water for steps 9, 11, and 13, as minerals dissolved in normal tap water contribute to the overall character of the stain.

MATERIALS TEXT

Hematoxylin, Richard Allen Scientific, Thermo Scientific, catalog #7211
Eosin Y, Richard Allen Scientific, Thermo Scientific, catalog # 7111
Clarifier, Richard Allen Scientific, Thermo Scientific, catalog # 7401
Bluing Reagent, Richard Allen Scientific, Thermo Scientific catalog # 7301
Cytoseal XYL, Thermo Scientific catalog # 8312-4
Leica Apperio ImageScope Pathology Slide viewing Software

SAFETY WARNINGS

Xylene and Ethanol are very volatile and well as potentially hazardous to inhale. Use a certified fume hood for all steps in this protocol.

BEFORE STARTING

Make sure all de-paraffinization reagents (xylene and Ethanol) are fresh.

Tissue Preparation

1 **De-Paraffization of slides**
Place slides in to oven at 60^{o} C for one hour

2 Place slides in to Fresh Xylene for 2 minutes, repeat

3 **Rehydration of sections**
100% Ethanol, 1 minute, repeat

4 90% Ethanol, 1 minute, repeat

5 80% Ethanol, 1 minute, repeat

6 70% Ethanol, 1 minute, repeat

7 Distilled Water, 2 minutes

8 **Staining Steps**
Hematoxylin 4 minutes

9 Rinse in running Tap water until clear

10 Clarifier 1, 30 seconds

11 Rinse in tap water for 30 seconds

12 Bluing reagent, 1 minute

13 Rinse in Tap water for 30 seconds

14 95% Ethanol 1 minute

15 Eosin 1 minute

16 **Dehydration steps**
100% Ethanol 1 minute, repeat

17 Xylene, 1 minute

18 Mount slides with 20 x 50 mm Coverslips using Cytoseal XYL, making sure that there are no air bubbles beneath the coverslip. Press the coverslip gently to remove an air bubbles that may have formed.

19 Let slides dry in a horizontal position in a fume hood for at least 1 hour.
Slides can be stored indefinitely at room temperature in a slide box

20 For illustration, digital storage and possible pathology review slides are scanned on the Leica Aperio slide scanner. The file output is .svs and can be viewed by downloading freely available software from Leica.