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Hematoxylin and Eosin Stain - FOR PARAFFIN-EMBEDDED TISSUE SECTIONS

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol details Hematoxylin and Eosin Stain for paraffin-embedded tissue sections.

Attachments

911-2361.pdf
81KB

Guidelines

Results:

1. Nuclei are stained blue.
2. Cytoplasm stained various shades of pink, identifying different morphologic tissue components.

Materials

Reagents

Stock Solutions:

1% stock Eosin Eosin Working Solution

	A	B
	Compound	**Quantity**
	Eosin Y	1 gm
	80% EtOH	100 ml

Eosin Working Solution

	A	B
	Compound	**Quantity**
	Stock Eosin	45 ml
	80% EtOH	135 ml
	Glacial Acetic Acid	900 ul

0.1% Acid Alcohol Solution

	A	B
	Compound	**Quantity**
	ddH2O	500 ml
	100% EtOH	500 ml
	HCl	1 ml

Epredia™ Shandon™ Harris Hematoxylin **Fisher Scientific Catalog #**6765001

Hematoxylin and Eosin Stain

21m

1 Deparaffinize slides and hydrate to ddH_2O:

	A	B
	Compound	**Time**
	Xylenes	2 × 5 min
	100% EtOH	2 × 1 min
	95% EtOH	1 min each
	80% EtOH	1 min each
	70% EtOH	1 min each
	ddH2O	1 min each

2 Filter undiluted Harris Hematoxylin and Eosin using Whatman #4 filter paper.

3 Immerse sections in filtered Harris Hematoxylin for 00:05:00 . 5m

4 Rinse in 2 changes of ddH_2O.

5 Differentiate in 0.1% acid alcohol solution:

	A	B
	Compound	**Time**
	EtOH fixed tissue	2 sec
	NBF fixed tissue	4 sec

6 Rinse in tap H_2O for 10- 00:15:00 or until sections turn bright blue. 15m

7 Immerse in Eosin for 00:01:00 . 1m

8 Briefly rinse in tap H_2O.

9 Dehydrate in:

	A	B
	Compound	**Time**
	95% EtOH	2 × 30 sec
	100% EtOH	2 × 1 min
	Xylene	2 × 5 min

10 Coverslip with Cytoseal.