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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
	ddH ₂ O	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 ddH₂O:

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
ddH ₂ O	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 ddH₂O.



- 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 H₂O 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 H₂O.



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.