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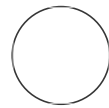
**Protocol status:** Working  
 We use this protocol and it's working

**Created:** Nov 30, 2023

# Hematoxylin and Eosin Stain - FOR PARAFFIN-EMBEDDED TISSUE SECTIONS

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## ABSTRACT

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

## ATTACHMENTS

[911-2361.pdf](#)

## 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

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**Funders**

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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 <sub>2</sub> 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 ddH<sub>2</sub>O:

A	B
Compound	Time
Xylenes	2 x 5 min
100% EtOH	2 x 1 min
95% EtOH	1 min each
80% EtOH	1 min each
70% EtOH	1 min each
ddH <sub>2</sub> 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 ddH<sub>2</sub>O.




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<sub>2</sub>O 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<sub>2</sub>O.



9 Dehydrate in:

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

**10** Coverslip with Cytoseal.