



Hematoxylin and Eosin Stain (H&E)

TECHNIQUE: Formalin fixed, paraffin tissue sections

REAGENTS:

Mayer's Hematoxylin (Fisher Scientific cat: NC9220898)

0.5% Acid Alcohol

70% Ethanol -----995ml
Hydrochloric Acid, (36.5% - 38%) ----- 5ml

1X PBS (pH 7.2-7.3)

Alcoholic-Eosin

1.0% Eosin Y

Eosin Y (CAS# 17372-87-1) ----- 1.0 g
Distilled water ----- 100 ml

1.0% Phloxine B

Phloxine B (CAS# 18472-87-2) ----- 0.5 g
Distilled water ----- 50.0 ml

Working Alcoholic-Eosin Solution

1.0% Eosin Y ----- 50.0 ml
1.0% Phloxine B ----- 5.0 ml
95% Ethanol ----- 390.0 ml
Acetic acid, glacial ----- 4.0 ml





Hematoxylin and Eosin Stain (H&E)

5 micron Paraffin Sections

PROCEDURE:

1. Deparaffinize and rehydrate slides to distilled water
2. Stain in **Mayers Hematoxylin** for **1 minute**
3. Wash with 4-5 changes of **Tap water** or until blue stops coming off slides
4. Blue nuclei in **1X PBS** for **1 minute**
5. Wash with 3 changes of **distilled water**
6. Counterstain in **Alcoholic-Eosin** for **1 minute (DO NOT RINSE)**
7. Dehydrate through **3 changes of 95% EtOH** and **2 changes of 100% EtOH 1 minute each**
8. Clear in **3 changes of Xylene 1 minute each change**
9. Mount and coverslip

*Do NOT rinse with water after Alcoholic-Eosin! Go directly into dehydration of slides.

RESULTS:

Nuclei ----- blue
Cytoplasm ----- pink
Erythrocytes ----- bright red
All other eosinophilic structures ----- red, pink, or orange





Hematoxylin and Eosin Stain For Fresh Frozen Section

(H&E)

PROCEDURE:

1. Take slides immediately from freezer and place in **cold fixative** (10% Neutral Buffered Formalin or 4% PFA) for **10 minutes**
2. Rinse slides in 1X PBS, 2 times for **3 minutes each** to remove OCT or other tissue embedding compound
3. Rinse in a gentle stream of **tap water** for **1 minute**
4. Stain in **Mayers Hematoxylin** for **30 seconds**
5. Wash in a gentle stream of **tap water** for **1 minute**
6. Blue nuclei in **1X PBS** for **20 seconds**
7. Wash in a gentle stream of **tap water** for **1 minute**
8. Dip in **70% EtOH** for **30 seconds**
9. Dip in **95% EtOH** for **30 seconds**
10. Counterstain in **Alcoholic-Eosin** for **30 seconds**
11. Dehydrate through 2 changes of **95% EtOH** and 3 changes of **100% EtOH** for **15 seconds each**
12. Clear through 3 changes of **Xylene** for **1 minute** each and coverslip

RESULTS:

Nuclei ----- blue
Cytoplasm ----- pink
Erythrocytes ----- bright red
All other eosinophilic structures ----- red, pink, or orange





Hematoxylin and Eosin Stain For Frozen Section

(H&E)

PROCEDURE:

1. Rinse slides in 1X PBS for **3 minutes**
2. Rinse in a gentle stream of **tap water** until OCT is washed off for **3 minutes**
3. Stain in **Mayers Hematoxylin** for **30 seconds**
4. Wash in a gentle stream of **tap water** for **1 minute**
5. Blue nuclei in **1X PBS** for **20 seconds**
6. Wash in a gentle stream of **tap water** for **1 minute**
7. Dip in **70% EtOH** for **30 seconds**
8. Dip in **95% EtOH** for **30 seconds**
9. Counterstain in **Alcoholic-Eosin** for **30 seconds**
10. Dehydrate through 2 changes of **95% EtOH** and 3 changes of **100% EtOH** for **15 seconds** each
11. Clear through 3 changes of **Xylene** for **1 minute** each and coverslip

RESULTS:

Nuclei ----- **blue**
Cytoplasm ----- **pink**
Erythrocytes ----- **bright red**
All other eosinophilic structures ----- **red, pink, or orange**





Hematoxylin and Eosin Stain for soft tissue

(H&E)

4 micron Paraffin Sections

PROCEDURE:

1. Deparaffinize and rehydrate slides to distilled water
2. Stain in **Mayers Hematoxylin** for **4 minutes**
3. Wash with 4-5 changes of **Tap water** or until blue stops coming off slides
4. Decolorize with 1 dips in **0.5% acid alcohol**
5. Wash in 3 changes of **distilled water**
6. Blue nuclei in **1X PBS** for **1 minute**
7. Wash with 3 changes of **distilled water**
8. Counterstain in **Alcoholic-Eosin** for **30 seconds (DO NOT RINSE)**
9. Dehydrate through **3 changes of 95% EtOH** and **2 changes of 100% EtOH 1 minute each**
10. Clear in **3 changes of Xylene 1 minute each change**
11. Mount and coverslip

*Do NOT rinse with water after Alcoholic-Eosin! Go directly into dehydration of slides.

RESULTS:

Nuclei ----- blue
Cytoplasm ----- pink
Erythrocytes ----- bright red
All other eosinophilic structures ----- red, pink, or orange

