

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

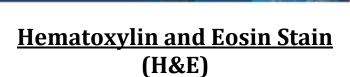
Phloxine B (CAS# 18472-87-2) ------ 0.5 g Distilled water ----- 50.0 ml

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 |







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 3changes of **100% EtOH** for **15 seconds each**
- 12. Clear through 3changes of **Xylene for 1 minute** each and coverslip

RESULTS:

| Nuclei | · blue |
|-----------------------------------|--------|
| Cytoplasm | pink |
| Erythrocytes | |
| All other eosinophilic structures | O |







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 3changes of **100% EtOH** for **15 seconds** each
- 11. Clear through 3changes of **Xylene for 1 minute** each and coverslip

RESULTS:

| Nuclei | · blue |
|-----------------------------------|--------------|
| Cytoplasm | pink |
| Erythrocytes | · bright red |
| All other eosinophilic structures | • |





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

