Hematoxylin and Eosin Stain
(H&E)

TECHNIQUE: Formalin fixed, paraffin tissue sections

REAGENTS:

Mayer’s Hematoxylin (Source Medical, catalog# 9235360)

0.5% Acid Alcohol

80% Ethanol -------------------------------------------------- 995 ml
Hydrochloric Acid, (36.5% - 38%) -------------------------------------- 5 ml
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 **Mayer's 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** for **15 seconds**
12. Dehydrate through 3 changes of **100% EtOH** for **15 seconds**
13. 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 for 15 seconds
11. Dehydrate through 3 changes of 100% EtOH for 15 seconds
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 soft tissue**  
*(H&E)*  
4 micron Paraffin Sections

**PROCEDURE:**

1. Deparaffinize and rehydrate slides to distilled water
2. Stain in **Mayer’s Hematoxylin** for **1 minute**
3. Wash with 4-5 changes of **Tap water** or until blue stops coming off slides
4. Decolorize with 3 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**