Alcian Blue Hematoxylin/ Orange G Stain for Bone and Cartilage

**TECHNIQUE:** Formalin fixed, paraffin tissue sections

**REAGENTS:**

- **Mayer’s Hematoxylin.** (Source Medical Products Catalog #9235360)
- **Alcian Blue Hematoxylin (3 months)**
  - Alcian Blue (CAS# 75881-23-1) ----------------------------------------------- 1 g
  - *Mayer’s Hematoxylin* (Source Medical Products Catalog #9235360) ---- 100 ml
- **Acid-Alcohol**
  - 70% Ethanol ----------------------------------------------- 500 ml
  - Hydrochloric acid, [36.5-38%] ------------------------------------------ 5 ml
- **0.5% Ammonium Water**
  - Ammonium Hydroxide, [28.0-30.0%] ---------------------------------- 2.5 ml
  - Distilled Water ------------------------------------------------------ 500 ml
Eosin/Orange G with Phloxine B

Stock Eosin (2 months)

Eosin Y (CAS# 17372-87-1) ----------------------------------------------- 0.6 g
Distilled Water -------------------------------------------------------- 50 ml
100% Ethanol ----------------------------------------------------------- 450 ml

Stir well until dissolved. Adjust the pH with glacial acetic acid until the pH is between 4.6 and 5.0

1% Phloxine B (make fresh)

Phloxine B (CAS# 18472-87-2) ----------------------------------------------- 0.4 g
Distilled Water -------------------------------------------------------- 40 ml

2% Orange G (make fresh)

Orange G (CAS# 1936-15-8) ----------------------------------------------- 0.4 g
Distilled Water -------------------------------------------------------- 20 ml

Working Eosin/Orange G Solution (2 months)

Stock Eosin --------------------------------------------------------------- 500 ml
1% Phloxine B ------------------------------------------------------------- 37 ml
2% Orange G --------------------------------------------------------------- 16 ml
**Alcian Blue Hematoxylin/ Orange G Stain**

Paraffin sections – 5 microns

**PROCEDURE:**

1. Deparaffinize slides and hydrate to **70% and AIR DRY**
2. Place in **Acid-alcohol** for **30 seconds** and drain briefly on paper towel (DO NOT RINSE)
3. Place in **Alcian Blue Hematoxylin** for **25 minutes**
4. Wash gently in **distilled water** for **4 changes**
5. Differentiate in **Acid-alcohol** for **3 seconds**
6. Rinse gently in **distilled water** with **3 changes**
7. Place in **0.5% Ammonium water** for **15 seconds**
8. Rinse in **distilled water** with **2 changes**
9. Place in **95% EtOH** for **1 minute** (DO NOT RINSE)
10. Place in **Eosin/ Orange G** for **1 min 30 sec**
11. Dehydrate with 3 changes of **95% EtOH** and 2 changes of **100% EtOH (1 minute per change)**
12. Clear with 3 changes of xylene and coverslip

**RESULTS:**

- Activated osteocytes -------------- bright blue pericellular ring
- Growth plate --------------------- pale blue to blue
- Cartilage ------------------------- blue/ purple (GAG/ proteoglycan)
- Bone ----------------------------- orange to red
- Erythrocytes ---------------------- bright pink
- Soft tissues (muscle, tendon, membranes) -- pink to red
- Bone marrow -------------------- dark blue
**Alcian Blue Hematoxylin/ Orange G Stain**  
**Fresh Frozen sections**

**PROCEDURE:**

1. Take slides immediately from freezer and place in **cold fixative** (10%Neutral Buffered Formalin or 4% PFA) for **10 minutes**
2. Wash slides in **1X PBS, 2 times for 5 minutes each**
3. Rinse in **distilled water, 3 times for 1 minute each**
4. Place in **Acid-alcohol** for **15 seconds** and drain briefly on paper towel (DO NOT RINSE)
5. Place in **Alcian Blue Hematoxylin** for **12 minutes**
6. Wash gently in **distilled water** for **4 changes**
7. Differentiate in **Acid-alcohol** for **1 dip**
8. Rinse gently in **distilled water** with **3 changes**
9. Place in **0.5% Ammonium water** for **15 seconds**
10. Rinse in **distilled water** with **2 changes**
11. Place in **95% EtOH** for **30 seconds** (DO NOT RINSE)
12. Place in **Eosin/ Orange G** for **30 seconds**
13. Dehydrate with 3 changes of **95% EtOH** and 2 changes of **100% EtOH** (15 seconds per change)
14. Clear with **3 changes of xylene for 1 minute each** and coverslip

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PROCEDURE:

1. Wash slides in **1X PBS, 2 times for 5 minutes** each
2. Rinse in **distilled water, 3 times for 1 minute each**
3. Place in **Acid-alcohol** for **15 seconds** and drain briefly on paper towel (DO NOT RINSE)
4. Place in **Alcian Blue Hematoxylin** for **12 minutes**
5. Wash gently in **distilled water** for **4 changes**
6. Differentiate in **Acid-alcohol** for **1 dip**
7. Rinse gently in **distilled water** with **3 changes**
8. Place in **0.5% Ammonium water** for **15 seconds**
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