



Alcian Blue Hematoxylin/ Orange G Stain for Bone and Cartilage

TECHNIQUE: Formalin fixed, paraffin tissue sections

REAGENTS:

Mayer's Hematoxylin (Fisher Scientific Cat: NC9220898)

Alcian Blue Hematoxylin (3 months)

Alcian Blue (CAS# 75881-23-1) ----- 1 g
Mayer's Hematoxylin ----- 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

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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

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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

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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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Activated osteocytes -----	bright blue pericellular ring
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Frozen sections

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**
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