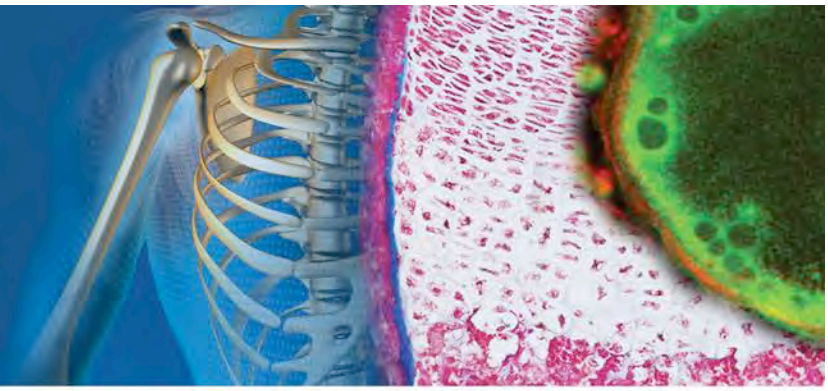


Alcian Blue Hematoxylin/ Orange G Stain for Bone and Cartilage

TECHNIQUE: Formalin fixed, paraffin tissue sections

REAGENTS:

Mayer's Acid Hematoxylin (6 months)

Hematoxylin (CAS# 517-28-2) ---------- **2.5 g**
Distilled Water ---------- **350 ml**
Aluminum Ammonium Sulfate (CAS# 7784-26-1) ---------- **25 g**
Sodium Iodate (CAS# 7681-55-2) ---------- **0.25 g**
Glycerol (CAS# 56-81-5) ---------- **150 ml**
Glacial Acetic acid (CAS# 64-19-7) ---------- **10 ml**

Dissolve 2.5 g Hematoxylin in 350 ml distilled water with stirring overnight. Next day add 25 g Aluminum Ammonium Sulfate and 0.25 g Sodium Iodate and stir overnight. Next day add glycerol and glacial acetic acid. Filter before use.

Alcian Blue Hematoxylin (3 months)

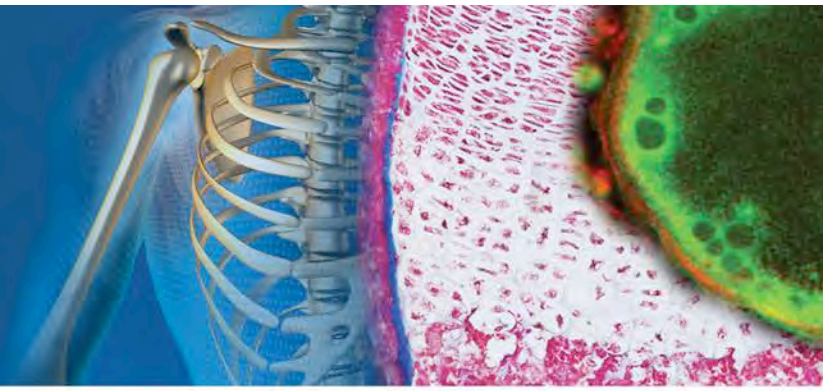
Alcian Blue (CAS# 75881-23-1) ---------- **1 g**
Mayer's Acid Hematoxylin ----------**100 ml**

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**



Eosin/Orange G with Phoxine 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)

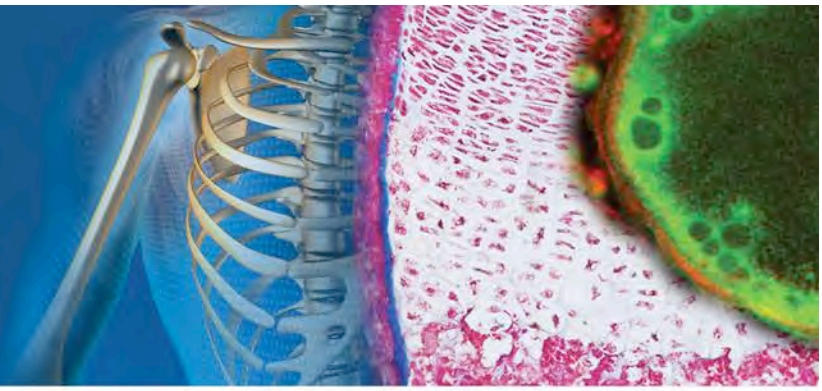
Phloxine B (CAS# 18472-87-2) ---------- **0.4 g**
Distilled Water ---------- **40 ml**

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 ---------- **16 ml**



Alcian Blue Hematoxylin/ Orange G Stain

(For excellent Growth Plate cartilage morphology)

Paraffin sections – 3 microns

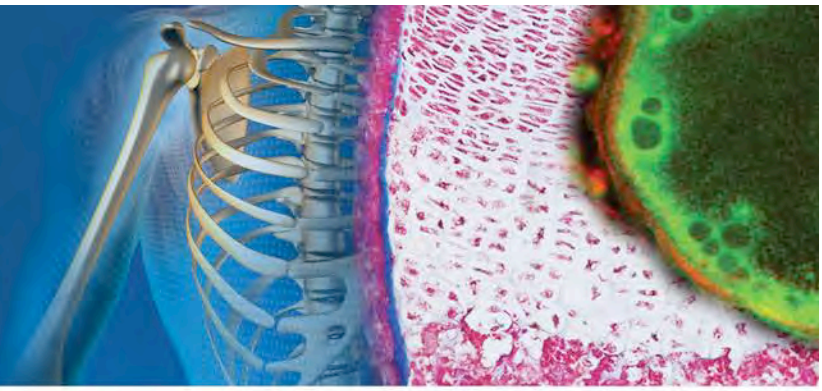
PROCEDURE:

1. Deparaffinize slides and hydrate to distilled water
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** until excess stain stops leaching from tissue (approximately 3 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

*Colors are more intense in Formic acid decalcified sections compared with EDTA decalcified sections



Alcian Blue Hematoxylin/ Orange G Stain

(For staining in both the Growth Plate cartilage and Articular Surface cartilage)

Paraffin sections – 3 microns

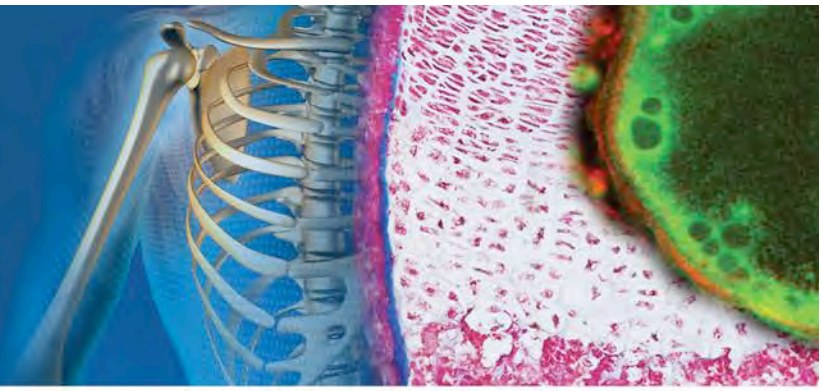
PROCEDURE:

1. Deparaffinize slides and hydrate to distilled water
2. Place in **Acid-alcohol** for **30 seconds** and drain briefly on paper towel (DO NOT RINSE)
3. Place in **Alcian Blue Hematoxylin** for **40 minutes**
4. Wash gently in **distilled water** until excess stain stops leaching from tissue (approximately 3 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 ---------- 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

*Colors are more intense in Formic acid decalcified sections compared with EDTA decalcified sections



Alcian Blue Hematoxylin/ Orange G Stain

(For staining in both the Growth Plate cartilage and Articular Surface cartilage)

EMBRYONIC Paraffin sections – 5 or 6 microns

PROCEDURE:

1. Deparaffinize slides and hydrate to distilled water
2. Place in **Acid-alcohol** for **20 seconds** and drain briefly on paper towel (DO NOT RINSE)
3. Place in **Alcian Blue Hematoxylin** for **10 minutes**
4. Wash gently in **distilled water** until excess stain stops leaching from tissue (approximately 3 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 minute**
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

*Colors are more intense in Formic acid decalcified sections compared with EDTA decalcified sections