Safranin O Staining Protocol for Cartilage

Description: This method is used for the detection of cartilage, mucin, and mast cell granules on formalin-fixed, paraffin-embedded tissue sections, and may be used for frozen sections as well. The cartilage and mucin will be stained orange to red, and the nuclei will be stained black. The background is stained bluish green.

Fixation: Formalin fixed, paraffin embedded sections.

Solutions and Reagents:

Weigert's Iron Hematoxylin Solution:

Stock Solution A:
Hematoxylin ---------- 1 g
95% Alcohol ---------- 100 ml

Stock Solution B:
29% Ferric chloride in water ---------- 4 ml
Distilled water ---------- 95 ml
Hydrochloric acid, concentrated ---- 1ml

Weigert's Iron Hematoxylin Working Solution:
Mix equal parts of stock solution A and B. This working solution is stable for about 4 weeks.

0.05% Fast Green (FCF) Solution:
Fast green, FCF, C.I. 42053 ---------- 0.5 g
Distilled water ---------- 1000 ml

1% Acetic Acid Solution:
Acetic acid, glacial ---------- 1 ml
Distilled water ---------- 99 ml

0.1% Safranin O Solution:
Safranin O, C.I. 50240 ---------- 0.1 g
Distilled water ---------- 100 ml

Procedure:

1. Deparaffinize and hydrate slides to distilled water.
2. Stain with Weigert's iron hematoxylin working solution for 10 minutes.
3. Wash in running tap water for 10 minutes.
4. Stain with fast green (FCF) solution for 5 minutes.
5. Rinse quickly with 1% acetic acid solution for no more than 10 –15 seconds.
6. Stain in 0.1% safranin O solution for 5 minutes.
7. Dehydrate and clear with 95% ethyl alcohol, absolute ethyl alcohol, and xylene, using 2 changes each, 2 minutes each.
8. Mount using resinous medium.

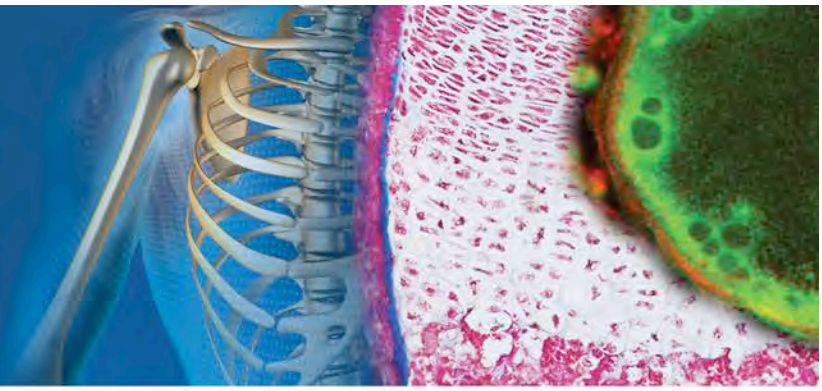
Results:

Nuclei ---------- black
Cytoplasm ---------- bluish green
Cartilage, mucin, mast cell granules ---------- orange to red

Find Images

References:

1. Kahveci Z, Minbay FZ, Cavusoglu L (2000) Safranin O staining using a microwave oven. Biotech Histochem. 75(6):264-8. PubMed Abstract
2. Tran D, Golick M, Rabinovitz H, Rivlin D, Elgart G, Nordlow B (2000) Hematoxylin and safranin O staining of frozen sections. Dermatol Surg. 26(3):197-9. PubMed Abstract
3. Camplejohn KL, Allard SA. Limitations of safranin 'O' staining in proteoglycan-depleted cartilage demonstrated with monoclonal antibodies. Histochemistry. 1988;89(2):185-8. PubMed Abstract



Safranin O/ Fast Green Stain for Cartilage

TECHNIQUE: Formalin fixed, paraffin tissue sections

REAGENTS:

Modified Weigert's Iron Hematoxylin

***Stock Solution A (*4 months)**

Hematoxylin (CAS# 517-28-2) ---------- **10.0 g**
80% EtOH ---------- **500.0 ml**

Stock Solution B **(4 months)**

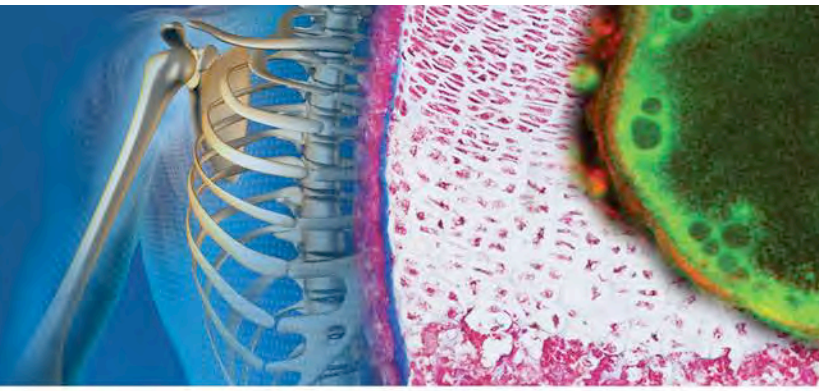
Ferric Chloride (CAS# 7705-08-0) ---------- **20.0 g**
Distilled water ---------- **475.0 ml**
Hydrochloric acid, [36.5-38.0%] ---------- **5.0 ml**

Working Modified Weigert's Iron Hematoxylin **(1 week)**

Mix equal parts of stock solutions A and B.

1.0% Acid-Alcohol

70% Ethanol ---------- **500.0 ml**
Hydrochloric acid, [36.5-38.0%] ---------- **5.0 ml**



0.02% Fast Green

Fast Green (CAS# 2353-45-9) ---------- **0.05 g**
Distilled water ---------- **250.0 ml**

1.0% Acetic Acid

70% Ethanol ---------- **100.0 ml**
Acetic acid, glacial ---------- **1.0 ml**

1.0% Safranin O

Safranin O (CAS# 477-23-6) ---------- **2.5 g**
Distilled water ---------- **250.0 ml**

Safranin O/ Fast Green Stain

(For excellent Growth Plate Cartilage morphology)

Paraffin sections- 3 microns

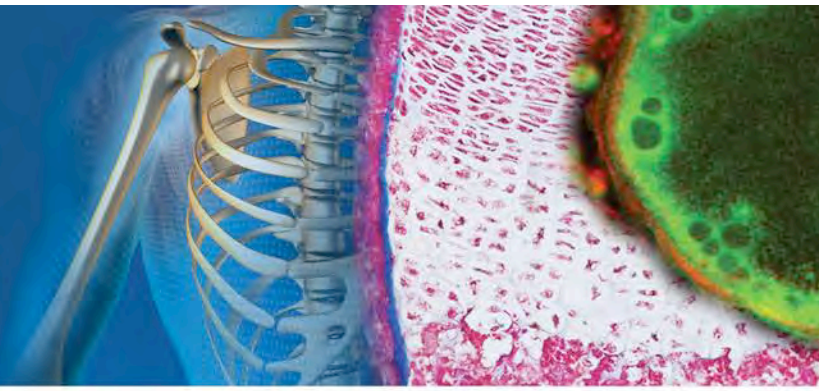
PROCEDURE:

1. Deparaffinize and hydrate to distilled water
2. Weigert's Iron Hematoxylin for **5 minutes**
3. Wash gently in distilled water, approximately **4 changes** or until excess dye stops leaching out of tissue
4. Differentiate in 1% Acid-Alcohol for **2 seconds**
5. Rinse gently in distilled water with **3 changes**
6. 0.02% Fast Green for **1 minute (do not rinse)**
7. 1.0% Acetic acid for **30 seconds (do not rinse)**
8. 1.0% Safranin O for **10 minutes (do not rinse)**
9. Rinse in 95% EtOH (briefly)
10. Dehydrate with 3 changes of 95% EtOH and 2 changes of 100% EtOH (1 minute per change)
11. Clear in 3 changes of Xylene and coverslip

RESULTS:

Cartilage (proteoglycan specific) ---------- red
Background ---------- green

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



Safranin O/ Fast Green Stain

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

Paraffin sections- 3 microns

PROCEDURE:

1. Deparaffinize and hydrate to distilled water
2. Weigert's Iron Hematoxylin for **5 minutes**
3. Wash gently in distilled water, approximately **4 changes** or until excess dye stops leaching out of tissue
4. Differentiate in 1% Acid-Alcohol for **2 seconds**
5. Rinse gently in distilled water with **3 changes**
6. 0.02% Fast Green for **1 minute (do not rinse)**
7. 1% Acetic acid for **30 seconds (do not rinse)**
8. 1.0% Safranin O for **30 minutes (do not rinse)**
9. Rinse in 95% EtOH (briefly)
10. Dehydrate with 3 changes of 95 % EtOH and 2 changes of 100% EtOH (1 minute per change)
11. Clear in 3 changes of Xylene and coverslip

RESULTS:

Cartilage (proteoglycan specific) ---------- red
Background ---------- green

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

Safranin-O Staining Protocol for Cartilage
Karen Lyons Lab - UCLA

I. Description: This method is used for the detection of cartilage, mucin, and mast cell granules on formalin-fixed, paraffin-embedded tissue sections, and may be used for frozen sections as well. The cartilage and mucin will be stained orange to red, and the nuclei will be stained black. The background is stained green.

II. Fixation: Formalin fixed, paraffin embedded sections.

III. Solutions and Reagents:

A) Hematoxylin QS Solution (Vector Laboratories, Inc., Cat# H3404)

B) 0.001% Fast Green (FCF) Solution:

Fast green, FCF, C.I. 42053 ---------- 0.01 g
Distilled water ---------- 1000 ml

C) 1% Acetic Acid Solution:

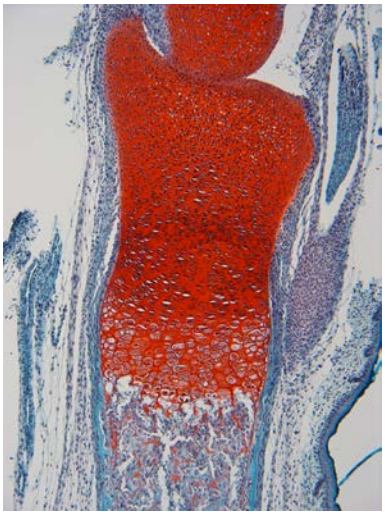
Acetic acid, glacial ---------- 2 ml
Distilled water ---------- 198 ml

D) 0.1% Safranin O Solution:

Safranin O, C.I. 50240 ---------- 0.1 g
Distilled water ---------- 100 ml

E) Acid EtOH

Concentrated HCl ----------500 ul
70% EtOH ----------200 ml



Safranin-O staining of distal tibia

Procedure:

1. Deparaffinize and hydrate slides to distilled water.
2. Stain with Hematoxylin QS solution for 5 minutes.
3. Wash in running tap water for 5 minutes.
4. Destain quickly in Acid EtOH (2-3 dips)
5. Wash in running tap water for 2x 1 minute.
6. Stain with fast green (FCF) solution for 5 minutes.
7. Rinse quickly with 1% acetic acid solution for no more than 10 –15 seconds.
8. Stain in 0.1% safranin O solution for 5 minutes.
9. Dehydrate and clear with 95% ethyl alcohol, absolute ethyl alcohol, and xylene: 2x, 2 min each.
10. Mount using resinous medium.

Results:

Nuclei ---------- black
Cytoplasm ---------- gray green
Cartilage, mucin, mast cell granules ---------- orange to red