



## 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



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## 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

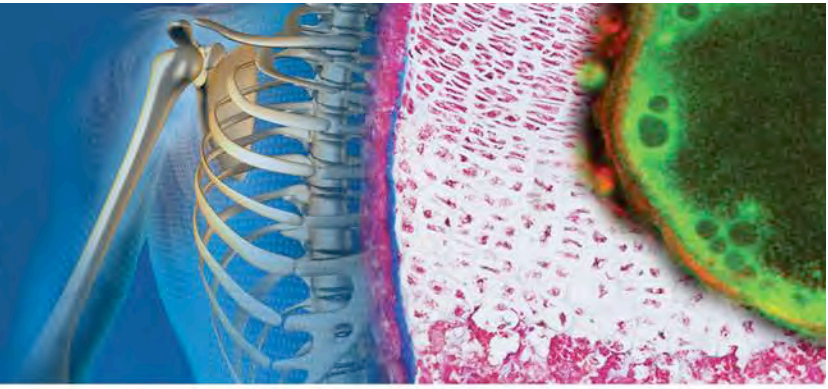
## 1.0% Safranin O

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



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MEDICINE of the HIGHEST ORDER



**Safranin O/ Fast Green Stain**  
*(For excellent Growth Plate Cartilage morphology)*

**Paraffin sections- 3 microns**

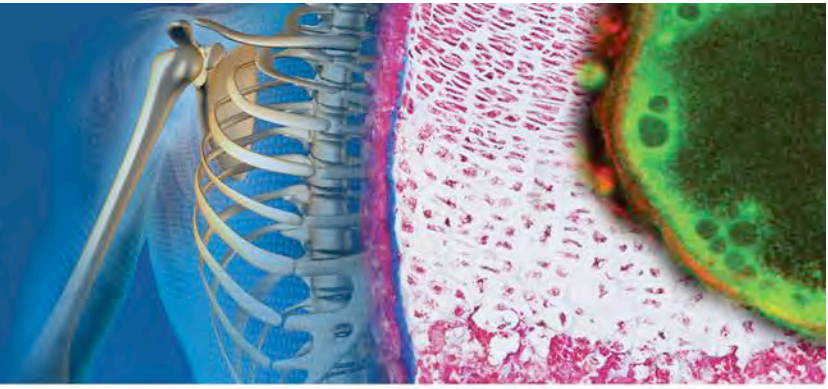
**PROCEDURE:**

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.

