**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)*

**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.*