



Safranin O/ Fast Green Stain for Cartilage

TECHNIQUE: Formalin fixed, Callis EDTA decalcified (Webb Jee method), paraffin embedded tissue sections

REAGENTS:

Modified Weigert's Iron Hematoxylin (Part A, EMS Catalog #26044-06, Part B EMS Catalog#26044-16)

Working Modified Weigert's Iron Hematoxylin (1 week, stored in dark)
Mix equal parts of stock solutions A and B.

1.0% Acetic Acid

Distilled water -----495 ml
Glacial acetic acid----- 5 ml

0.5% Acetic Acid

Distilled water ----- 497.5 ml
Glacial acetic acid----- 2.5 ml

0.08% Fast Green

Fast Green (CAS# 2353-45-9) ----- 0.20 g
Distilled water ----- 250 ml

0.1% Safranin O

Safranin O (CAS# 477-23-6) ----- 0.25 g
Distilled water ----- 250 ml



Safranin O/ Fast Green Stain
(For excellent Growth Plate Cartilage morphology)
Paraffin sections- 3-5 microns

PROCEDURE:

1. Bake slides at 60°C overnight; cool at room temperature 20-30 min.
2. Deparaffinize and hydrate to 70% ethanol and **air dry**
3. Stain with Weigert's Iron Hematoxylin for **7 minutes**
4. Wash gently in running Tap Water **10 minutes**
5. Stain with 0.08% Fast Green for **3 minutes (blot, no rinse)**
6. Rinse in 1.0% Acetic acid for **10 seconds (blot, no rinse)**
7. Stain with 0.1% Safranin O for **5 minutes (blot, no rinse)**
8. Single dip in 0.5% Acetic acid *To minimize cartilage folding/ flipping
9. Rinse carefully in two changes of distilled water and **air dry**
10. Clear in 1 dip of Xylene and coverslip *To minimize cartilage folding/ flipping

RESULTS:

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

REFERENCES:

Lillie RD, Fulmer HM. *Histopathologic Technic and Practical Histochemistry*, 4th ed. New York, NY: McGraw-Hill; 1976:657.
Conn HJ, Lillie RD. *Biological Stains*, 9th ed. Baltimore, MD: Waverly Press; 1977: 387.