



Safranin O/ Fast Green Stain for Cartilage

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

REAGENTS:

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

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

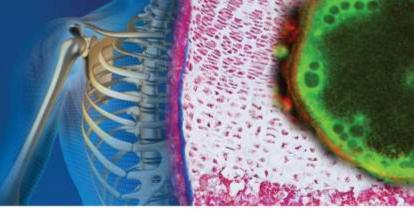
Fast Green (CAS# 2353-45-9)	0.20 g
Distilled water	250 ml

0.1% Safranin 0

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.

