

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

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](#)