Safranin-O Staining Protocol for Cartilage
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I. 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 green.

II. Fixation: Formalin fixed, paraffin embedded sections.

III. Solutions and Reagents:

A) Hematoxylin QS Solution (Vector Laboratories, Inc., Cat# H3404)

B) 0.001% Fast Green (FCF) Solution:

Fast green, FCF, C.I. 42053 ---------- 0.01 g
Distilled water ---------- 1000 ml

C) 1% Acetic Acid Solution:

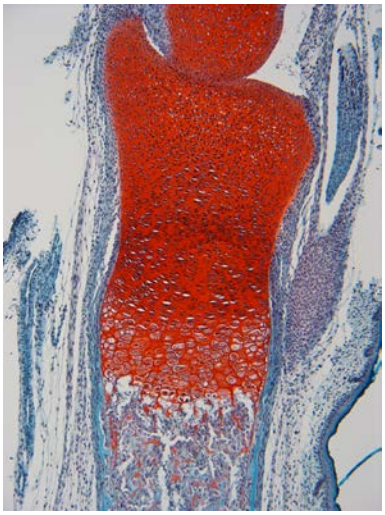
Acetic acid, glacial ---------- 2 ml
Distilled water ---------- 198 ml

D) 0.1% Safranin O Solution:

Safranin O, C.I. 50240 ---------- 0.1 g
Distilled water ---------- 100 ml

E) Acid EtOH

Concentrated HCl ----------500 ul
70% EtOH ----------200 ml



Safranin-O staining of distal tibia

Procedure:

1. Deparaffinize and hydrate slides to distilled water.
2. Stain with Hematoxylin QS solution for 5 minutes.
3. Wash in running tap water for 5 minutes.
4. Destain quickly in Acid EtOH (2-3 dips)
5. Wash in running tap water for 2x 1 minute.
6. Stain with fast green (FCF) solution for 5 minutes.
7. Rinse quickly with 1% acetic acid solution for no more than 10 –15 seconds.
8. Stain in 0.1% safranin O solution for 5 minutes.
9. Dehydrate and clear with 95% ethyl alcohol, absolute ethyl alcohol, and xylene: 2x, 2 min each.
10. Mount using resinous medium.

Results:

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