ScyTek LABORATORIES INC.

Instructions For Use

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Safranin O Stain Kit for Cartilage

Description and Principle

Safranin O (0.1%) is commonly used to demonstrate Glycosaminoglycagns (GAGs) in FFPE cartilage sections. Weigert's Hematoxylin is provided to stain nuclei and Fast Green FCF for bluish green background staining.
Safranin O is a basic dye that binds with a high affinity to acidic proteoglycans in cartilage. Dilute fast green FCF is used to counterstain and nuclei are stained by a rapid iron mordanted hematoxylin solution.

Expected Results

Glycosaminoglycans:	Pink to Red
Background:	Bluish Green
Nuclei:	Blue to Black

Kit Contents

	Storage
1. Safranin O (0.1%)	18-25°C
2. Hematoxylin, Weigert's Iron (Part A)	18-25°C
3. Hematoxylin, Weigert's Iron (Part B)	18-25°C
4. Fast Green FCF (0.1%)	18-25°C
5. Acetic Acid Solution 1%	18-25°C.

Suggested Controls *(not provided)*

Articular Cartilage

Uses/Limitations

For Research Use Only.
Do not use if reagents become cloudy or precipitate
Do not use past expiration date.
Use caution when handling reagents.
Non-Sterile
Intended for FFPE sections cut at 5-10µm.
This procedure has not been optimized for frozen sections.
Frozen sections may require protocol modification.

Storage

Store kit and all components at room temperature (18-25°C).

Safety and Precautions

Please see current Safety Data Sheets (SDS) for this product and components GHS classification, pictograms, and full hazard/precautionary statements.

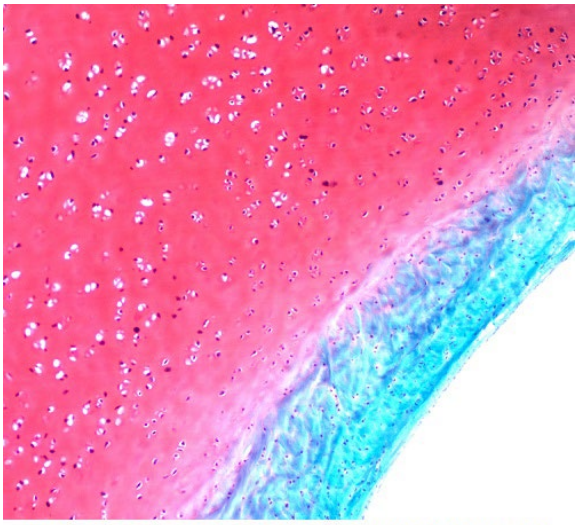
Important Notes:

1. Although Fast Green FCF is supplied as a counterstain, there are some sources that suggest it binds competitively against Safranin O[1]. The counterstain my be omitted from the procedure if preferred.

2. Binding of Safranin O to GAGs may be stoichiometric when the levels of GAGs are not too low, therefore may not be a sensitive indicator for severely diseased cartilage.[2]

3. For comparative studies, standardization of fixation solution, stain time, temperature, pH, osmolarity, etc. is critical for apparent proteoglycan level and subsequent safranin staining.[3] Some acid fixatives and decalcification procedures may reduce levels of proteoglycans as well.

4. Safranin O (0.1%) is lightly buffered to pH 5.2 with acetate/acetic acid. Fast Green FCF is provided at a concentration of 0.1% but should be diluted for preferred and optimal staining. We've found a 0.05% working solution to be satisfactory (1:1 dilution with deionized water).



Avian Articular Cartilage stained with Safranin O Stain Kit for Cartilage

Procedure

1. Deparaffinize sections if necessary and hydrate to distilled water.

2. Mix equal volumes (1:1) of Hematoxylin Weigert's Iron parts A and B. Use immediately and dispose of after use (do not re-use). Apply to tissue and stain for 2-5 minutes.

3. Rinse slide in tap water for at least 2 minutes followed by deionized water.

4. (OPTIONAL) Dilute Fast Green FCF 1:1 with deionized water and stain for 2-5 minutes (*See note 4 above)*. Rinse stain off slide with Acetic Acid Solution 1% followed by a rinse in deionized water.

5. Apply Safranin O (0.1%) for 5-15 minutes.

6. Rinse slide briefly with absolute alcohol then quickly dehydrate slide in absolute alcohol.

7. Clear with xylene or substitute and mount in synthetic resin.

References

1. Bulstra SK, Drukker J, Kuijer R, Buurman WA, vander-Linden AJ. Thionin staining of paraffin and plastic embedded sections of cartilage. Biotech Histochem1993;68(1):20–8.
2. Camplejohn KL, Allard SA. Limitations of safranin 'O' staining in proteoglycan-depleted cartilage demonstrated
with monoclonal antibodies. Histochemistry 1988;89(2):185–8.
3. Hyllested JL, Veje K, Ostergaard K. Histochemical studies of the extracellular matrix of human articular cartilage--a review. Osteoarthritis Cartilage. 2002 May;10(5):333-43. doi: 10.1053/joca.2002.0519. PMID: 12027534.

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