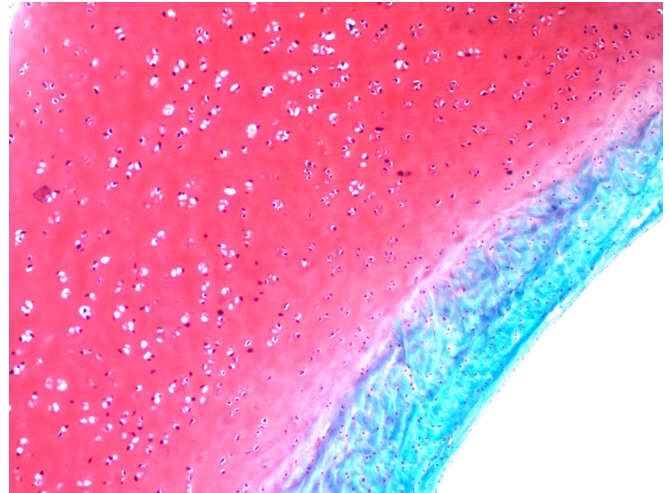


Safranin O Stain Kit for Cartilage

Description: Safranin O (0.1%) is commonly used to demonstrate Glycosaminoglycans (GAGs) in FFPE cartilage sections. Weigert's Hematoxylin is provided to stain nuclei and Fast Green FCF for bluish green background staining.

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



Uses/Limitations: Not to be taken internally.
For Research Use Only.
Do not use if reagents become cloudy.
Do not use past expiration date.
Use caution when handling reagents.
Non-Sterile.


Control Tissue: Articular Cartilage


Kit Contents:

<u>Item #</u>	<u>Kit Contents</u>	<u>Storage</u>
SON	Safranin O (0.1%)	18-25°C.
HWI-A	Hematoxylin Weigert's Iron A	18-25°C.
HWI-B	Hematoxylin Weigert's Iron B	18-25°C.
FCF	Fast Green FCF (0.1%)	18-25°C.
AAE	Acetic Acid 1%	18-25°C.

Important Notes:

1. Although Fast Green FCF is supplied as a counterstain, there are some sources that suggest it binds competitively against Safranin O¹. The counterstain may be omitted in the procedure if preferred.
2. Binding of Safranin O to GAGs may be stoichiometric when the levels of GAG 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 proteoglycan as well.

Storage: 18° C  25° C

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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 0.05% working solution to be satisfactory (1:1 dilution with deionized water).

Procedure (Standard):

1. Deparaffinize and hydrate to water through graded alcohols.
2. Mix equal parts (1:1) 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. Then rinse in 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 1% followed by a rinse in deionized water.
6. Apply Safranin O (0.1%) for 5-15 minutes.
7. Rinse slide briefly with absolute alcohol followed by quick dehydration in absolute alcohol.
8. Clear in xylene or substitute and mount in synthetic resin.

References:

1. Bulstra SK, Drukker J, Kuijjer R, Burman WA, vander-Linden AJ. Thionin staining of paraffin and plastic embedded sections of cartilage. *Biotech Histochem* 1993;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.

Storage: 18° C  25° C