



**Safranin O Staining Kit
(SafraninO)
Catalog #8348**

Product Description

Safranin O, an indicator of cell chondrogenesis, is a cationic dye that stains acidic proteoglycan present in cartilage tissues. The Safranin O Staining Kit contains 0.1g of Safranin O Stain in powder, which can easily be dissolved in deionized water to make the staining solution. Safranin O binds to glycosaminoglycan and shows an orange-red color [1].

Kit Components

Cat. No.	# of vials	Name	Quantity	Storage
8348a	5	Safranin O Stain	20 mg	Room temperature
8348b	5	Fast Green FCF	20 mg	Room temperature
8348c	1	1% Acetic Acid	100 mL	Room temperature
8348d	1	Xylene Substitute	100 mL	Room temperature

Materials Supplied by User

Formaldehyde-fixed and paraffin-embedded tissue sections
Ethanol (100%, 95%, 70%, 50%)
Deionized H₂O (diH₂O)

Product use

SafraninO is for research use only. It is not approved for human or animal use, or for application in clinical or *in vitro* diagnostic procedures.

Shipping

Room temperature.

References

[1] Mackay, A. M., Beck, S. C., Murphy, J. M., Barry, F. P., Chichester, C. O., & Pittenger, M. F. (1998). Chondrogenic differentiation of cultured human mesenchymal stem cells from marrow. *Tissue Engineer.* 4:415-428.

Procedures

A. Preparation of Safranin O and Fast Green staining solution

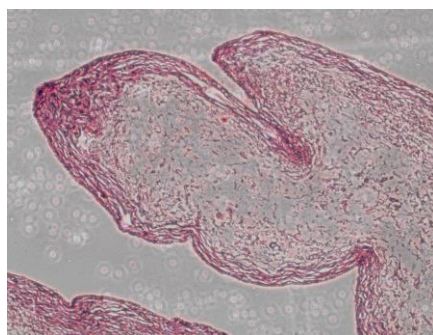
1. Transfer 20 mg of Safranin O Stain (Cat. #8348a) in one vial into a 100 mL beaker.
2. Add 20 mL of diH₂O into the beaker and dissolve the stain by stirring to make 0.1% Safranin O staining solution.
3. Transfer 20 mg of Fast Grene FCF (Cat. #8348b) in one vial into another 100 mL beaker.

4. Add 20 mL of diH₂O into the beaker and dissolve the stain by stirring to make 0.1% Fast Green solution.
5. Filter the Safranin O and Fast Green staining solution using a Nalgene PES 75mm filter.

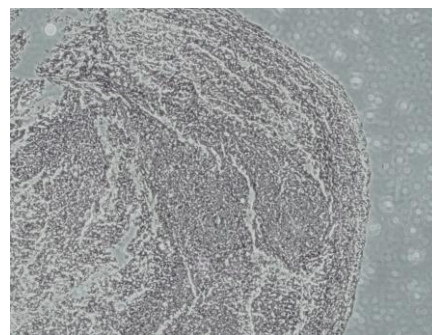
Note: It is recommended that the Safranin O solution be used within a month.

B. Preparation of tissue section slides

1. Deparaffinize and hydrate slides:
 - 1) Deparaffinize the tissue sections in Xylene Substitute (Cat. #8348d), 3 changes of 5 min per change.
 - 2) Hydrate in 100% ethanol, 2 changes of 2 min per change.
 - 3) Hydrate in 95% ethanol, 2 changes of 2 min per change.
 - 4) Hydrate in 70% ethanol for 2 min.
 - 5) Hydrate in 50% ethanol for 15 min.
 - 6) Wash in running tap water for 10 min.
2. Stain in 0.1% Fast Green Solution for 5-10 minutes.
3. Rinse in 1% Acetic Acid (Cat. #8348c) for 10-15 seconds.
4. Stain in 0.1% Safranin O staining solution for 20-30 min.
5. Dehydrate and clear slides:
 - 1) Dehydrate in 95% ethanol, 2 changes of 2 min per change.
 - 2) Dehydrate in 100% ethanol, 2 changes of 2 min per change.
 - 3) Clear the tissue sections in Xylene Substitute (Cat #8348d), 2 changes of 2 min per change.
6. Mount the tissue sections and observe under microscope.



(a)



(b)

Figure 1. (a) Human Dermal Fibroblasts-fetal (HDF-f, Cat. #2300) were cultured as pellets in growth medium, complete Fibroblast Medium (FM, Cat. #2301) for 50 days. The pellets were fixed in 4% paraformaldehyde and sectioned. Safranin O staining was not detected (Magnification: 10X).

(b) HDF-f were cultured as pellets in complete MSC Chondrogenic Differentiation Medium (MCDM, Cat. #7551) for 50 days. The pellets were fixed in 4% paraformaldehyde and sectioned. Safranin O staining demonstrated the presence of cartilage in cells (Magnification: 10X).