

Product Description

Safranin O is a cationic dye that stains acidic proteoglycan present in cartilage tissues, which are indicators cell chondrogenesis. This 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

This kit is for research use only. Not for use in animals, humans, or 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 (# 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 (# 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 (# 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.

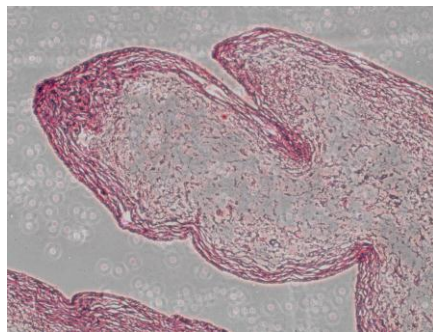
3. Rinse in 1% Acetic Acid (# 8348c) for 10-15 seconds.

4. Stain in 0.1% Safranin O staining solution for 20-30 min.

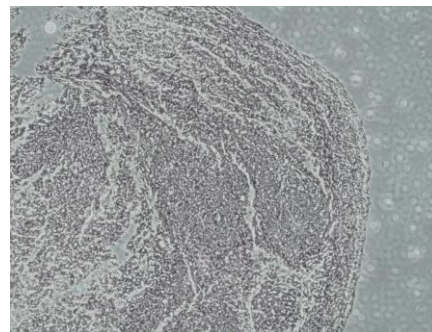
5. Dehydrate and clear slides:

- 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 (# 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, Catalog # 2300) were cultured as pellets in growth medium, complete Fibroblast Medium (FM, Catalog # 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, Catalog # 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).