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

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th># of vials</th>
<th>Name</th>
<th>Quantity</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>8348a</td>
<td>5</td>
<td>Safranin O Stain</td>
<td>20 mg</td>
<td>Room temperature</td>
</tr>
<tr>
<td>8348b</td>
<td>5</td>
<td>Fast Green FCF</td>
<td>20 mg</td>
<td>Room temperature</td>
</tr>
<tr>
<td>8348c</td>
<td>1</td>
<td>1% Acetic Acid</td>
<td>100 mL</td>
<td>Room temperature</td>
</tr>
<tr>
<td>8348d</td>
<td>1</td>
<td>Xylene Substitute</td>
<td>100 mL</td>
<td>Room temperature</td>
</tr>
</tbody>
</table>

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

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 Green 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.

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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).