Sirius Red/Fast Green Collagen Staining Solution

Catalog # 90463

For Research Use Only - Not Human or Therapeutic Use

INTRODUCTION

Sirius Red is combined with Fast Green for histochemical staining and the quantification of collagens and total proteins. This dual staining enables the clear distinction of collagen from surrounding materials through microscopic image analysis.

Sirius Red is widely used due to its specific reactivity with the [Gly-X-Y]n helical structure of fibrillar collagens, regardless of collagen type or species. Therefore, the dye is extensively employed for the quantitative estimations of collagen production in cultured cells and for evaluating tissue fibrosis in organs such as the liver, lung, kidney, and gastrointestinal tract.

Fast Green binds to non-collagenous proteins through electrostatic interactions between the negatively charged dye molecules and positively charged proteins.

Exploiting the unique features of these two dyes, Chondrex, Inc. offers a simple Sirius Red/Fast Green Collagen Staining Solution to visualize collagen and non-collagenous proteins in cultured cell layers and tissue sections from various tissues (1-7).

COMPONENTS

Item	Quantity	Amount	Storage
Sirius Red/Fast Green Dye Solution (90463)	1 bottle	250 ml	RT

PROTOCOL

The following sample preparation methods are standard protocols. Therefore, these protocols may need to be optimized depending on the sample types.

A. Paraffin Embedded Tissue Sections

1. Prepare paraffin-embedded tissue sections (approximately 30-50 mm^2, 10-20 µm thick).
2. Deparaffinize the tissue sections with the following steps below:
    1. Xylene, 10 minutes
    2. Xylene 1:1 with 100% ethanol, 10 minutes
    3. 100% ethanol, 10 minutes
    4. 50% ethanol/distilled water, 5 minutes
    5. Distilled water, 5 minutes
3. Transfer individual slides to petri dishes.
4. Load 0.2 - 0.3 ml Dye Solution on each sample, enough to completely immerse the tissue section, and incubate at room temperature for 30 minutes.

   NOTE: To avoid evaporation of the Dye Solution, place a piece of wet filter paper beneath the slide and cover the petri dish with a lid.
5. Carefully aspirate the Dye Solution.
6. Rinse the stained tissue section with 0.5 ml of distilled water repeatedly until the water runs clear.
7. Observe under a microscope. The stained samples can be dehydrated in 100% ethanol and then washed with xylene before being mounted in a resinous medium.

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B. Frozen Tissue Sections

1. Prepare frozen tissue sections (approximately 30-50 mm^2, 10-20 µm thick) according to standard methods.
2. Wash with 1X PBS.

   Optional fixing step (8,9): Add 0.5 ml of Kahle fixative, enough to completely immerse each tissue section, and incubate for 10 minutes at room temperature.

   Kahle fixative recipe

   - 60 ml distilled water
   - 28 ml 95-100% ethanol
   - 10 ml 37% formaldehyde
   - 2 ml glacial acetic acid.
3. Remove the fixative, wash with 1X PBS, and transfer individual slides to petri dishes.
4. Load 0.2 - 0.3 ml of Dye Solution on each sample, enough to completely immerse the tissue section, and incubate at room temperature for 30 minutes.

   NOTE: To avoid evaporation of the Dye Solution, place a piece of wet filter paper beneath the slide and cover the petri dish with a lid.
5. Carefully aspirate the Dye Solution.
6. Rinse the stained tissue section with 0.5 ml of distilled water repeatedly until the water runs clear.
7. Observe under a microscope. The stained samples can be dehydrated in 100% ethanol and then washed with xylene before being mounted in a resinous medium.

C. In Vitro Cultured Cell Layers

1. If planning to mount cell layers and observe with a microscope before proceeding with the dye extraction step, place a sterilized round glass slide at the bottom of each well of a 24-well culture plate. Otherwise, skip this step.
2. Culture cells in the 24-well culture plates for the required time.
3. Remove the culture media and wash the wells with 1X PBS.
4. Add 0.5 ml of Kahle fixative, enough to completely immerse the cell layers, and incubate for 10 minutes at room temperature.

   Kahle fixative recipe

   - 60 ml distilled water
   - 28 ml 95-100% ethanol
   - 10 ml 37% formaldehyde
   - 2 ml glacial acetic acid
5. Remove the fixative and wash with 1X PBS.
6. Add 0.2 - 0.3 ml of Dye Solution, enough to completely immerse the fixed cell layers, and incubate at room temperature for 30 minutes. If cells are on glass slides, remove the slides and place them in petri dishes for the staining.

   NOTE: To avoid evaporation of the Dye Solution, place a piece of wet filter paper beneath the slide and cover the petri dish with a lid.
7. Carefully aspirate the Dye Solution.
8. Rinse the stained cell layers with 0.5 ml of distilled water repeatedly until the water runs clear.
9. Observe under a microscope. The stained samples can be dehydrated in 100% ethanol and then washed with xylene before being mounted in a resinous medium.

TROUBLESHOOTING

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.'s ELISA FAQ for more information.

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