# Sirius Red/Fast Green Collagen Staining Kit

Catalog # 9046

*For Research Use Only - Not Human or Therapeutic Use*

## PRODUCT SPECIFICATIONS

| | |
|---|---|
| DESCRIPTION: | Stain kit to quantify total collagen |
| FORMAT: | Glass slides and tissue culture plates |
| ASSAY TYPE: | Dye staining |
| ASSAY TIME: | Varies depending on sample type |
| STANDARD RANGE: | Collagen: 3 µg/section |
| | Non-collagenous proteins: 50 µg/section |
| NUMBER OF SAMPLES: | 30-50 samples/kit |
| SAMPLE TYPES: | Cultured cells and tissue sections |
| RECOMMENDED SAMPLE DILUTIONS: | N/A |
| CHROMOGEN: | N/A (read at 540 and 605 nm) |
| STORAGE: | Room temperature for 12 months |
| VALIDATION DATA: | N/A |
| NOTES: | |

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## INTRODUCTION

Sirius Red and Fast Green is a dye combination used to distinguish collagen from its surrounding materials. Sirius Red specifically binds the $[Gly\text{-}X\text{-}Y]_n$ helical structure of fibrillar collagens, regardless of collagen type or species, whereas Fast Green binds to non-collagenous proteins. By exploiting the unique features of these two dyes, Chondrex, Inc. provides a simple semi-quantitative assay kit to determine the amounts of collagen and non-collagenous proteins in cultured cell layers and tissue sections (1-2). Because this assay does not require collagen solubilization, it can be used to measure total collagen content in various tissues (3-7).

As Sirius Red and Fast Green have absorptions at 540 nm and 605 nm respectively, the OD values of the extracted dyes can be used to calculate the collagen and non-collagenous protein content of samples. For general histological studies in which tissue sections are 10-20 μm thick, the assay sensitivity for collagen and non-collagenous proteins is greater than 3 μg/section and 50 μg/section, respectively. This kit contains enough reagents to stain 30-50 samples.

## KIT COMPONENTS

| Item | Quantity | Amount | Storage |
|---|---|---|---|
| Dye Solution (90461) | 1 bottle | 10 ml | RT |
| Dye Extraction Buffer (90462) | 2 bottles | 30 ml | RT |

## ASSAY 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

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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.

   OPTION: These samples may be observed under a microscope without the following extraction step. Dehydrate in 100% ethanol, followed by a xylene wash, and mount in a resinous medium.

7. Load 1 ml of Dye Extraction Buffer on each sample and gently mix by pipetting until the color is eluted from the tissue section.

8. Collect the eluted Dye Solution and read the OD values at 540 nm and 605 nm with a spectrophotometer.

**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.

   OPTION: These samples may be observed under a microscope without the following extraction step. Dehydrate in 100% ethanol, followed by a xylene wash, and mount in a resinous medium.

7. Load 1 ml of Dye Extraction Buffer on each sample and gently mix by pipetting until the color is eluted from the tissue section.

8. Collect the eluted Dye Solution and read the OD values at 540 nm and 605 nm with a spectrophotometer.

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**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.

   OPTION: These samples may be observed under a microscope without the following extraction step. Dehydrate in 100% ethanol, followed by a xylene wash, and mount with a resinous medium.

9. Load 1 ml of Dye Extraction Buffer on each sample and gently mix by pipetting until the color is eluted from the sample.

10. Collect the eluted dye solution and read the OD values at 540 nm and 605 nm with a spectrophotometer.

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## CALCULATING RESULTS

To calculate the amount of collagen, correct the OD 540 value by subtracting the contribution of Fast Green at 540 nm, which is 29.1% of the OD 605 value. The color equivalence (OD values/µg protein) is 0.0378 for collagen and 0.00204 for non-collagenous proteins at OD 540 and 605, respectively (2).

$$\text{Collagen (µg/section)} = \frac{\text{OD 540 value} - (\text{OD 605 value} \times 0.291)}{0.0378}$$

$$\text{Non-collagenous proteins (µg/section)} = \frac{\text{OD 605 value}}{0.00204}$$

NOTE 1: Thicker sections tend to detach from slides during washing steps. Please wash the slides carefully. Moreover, as the assay sensitivity depends on the surface area of the tissue on the slides, cutting thinner sections will not significantly increase the assay sensitivity.

NOTE 2: Non-collagenous protein values may be used to normalize the results of samples because protein levels are relative to the surface area or cell density of the samples.

## TROUBLESHOOTING

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

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