# Sirius Red Collagen Detection Kit

Catalog # 9062

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

## INTRODUCTION

Sirius red is a unique dye which specifically binds to the $[Gly\text{-}X\text{-}Y]_n$ helical structure on fibrillar collagen (type I to V) and does not discriminate between collagen species and types. Therefore, this kit is designed to detect the total collagen content in various samples.

Chondrex, Inc. provides a Sirius Red Collagen Detection Kit for various collagen-containing samples such as tissue specimens, cell culture media, and cultured cells. The total assay working time is less than 30 minutes and 40 samples can be measured in duplicate. Due to the low level of collagen in cell culture media, additional concentration steps may be necessary.

For determining levels of collagen from individual species or different types of samples, we recommend our Type I Collagen Detection kits (Catalog # 6012-6016, 6019, 6021) and Type II Collagen Detection Kit (catalog # 6018). For convenience, the same sample preparations may be used for both the Collagen Detection ELISA Kits and the Sirius Red Collagen Detection Kit.

## KIT COMPONENTS

| Item | Quantity | Amount | Storage |
|---|---|---|---|
| Standard - Bovine Type I Collagen (90621) | 1 vial | 0.5 mg/ml, 1 ml | -20°C |
| Sirius Red Solution (90622) | 1 bottle | 50 ml | -20°C |
| Washing Solution (90623) | 1 bottle | 50 ml | -20°C/* |
| Extraction Solution (90624) | 1 bottle | 30 ml | -20°C/* |
| 0.5M Acetic Acid (10X Acetic Acid) (90625) | 1 bottle | 20 ml | -20°C/* |
| 96-Well Plate (9026) | 1 each | 8-well strips x 12 | -20°C/* |

*Washing solution, extraction solution, 0.5M acetic acid, and the plate can also be stored at room temperature.

Concentrating Solution (50 ml, Catalog # 90626) for cell culture media samples is NOT included. Please contact Customer Service (support@chondrex.com) to place an order.

## SAMPLE PREPARATION

Tissue specimens and cultured cells can be used; however, solid samples must be solubilized for this assay. In addition, culture media samples may require a concentration process (please see page 3 of this protocol). Also, heat-denatured collagen tends to have a lower binding affinity for sirius red, resulting in underestimated values.

Depending on the solubilization method, these soluble collagen samples can be used:

1) Salt soluble collagen (0.15M NaCl in 0.1M Tris-HCl, pH 7.4)
2) Acid soluble collagen (0.05M acetic acid)
3) Pepsin soluble collagen (0.05M acetic acid with pepsin)

We recommend our “Tips For Collagen Solubilization” to prepare pepsin-soluble collagen samples. Please contact Customer Service (support@chondrex.com) for more information.



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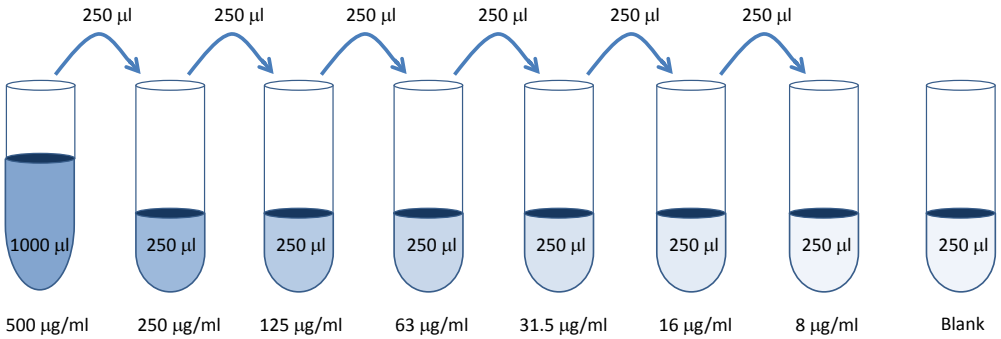
## ASSAY PROCEDURES (assay should be run in duplicate)

1) Using highly purified distilled water, prepare enough of a 1X acetic acid (0.05M) solution for the standards and your samples.

2) Prepare standard solutions in 1.5 ml centrifuge tubes or disposable culture tubes: add 250 µl of 0.05M acetic acid (Blank) to seven tubes. Mix 250 µl of Standard (500 µg/ml) with an equal amount of 0.05M acetic acid (250 µg/ml). Repeat this procedure five times to make 125, 63, 31.5, 16, and 8 µg/ml solutions.




3) Prepare sample solutions in 1.5 ml centrifuge tubes or disposable culture tubes. Since the collagen concentration of the sample is unknown, preparing multiple samples with varying dilutions using 0.05M acetic acid is recommended.

4) Add 100 µl of Blank, diluted standard solutions, and samples to 1.5 ml centrifuge tubes in duplicate.

5) Add 500 µl of Sirius Red Solution to each tube.

6) Vortex and incubate for 20 minutes at room temperature.

7) Centrifuge at 10,000 rpm for 3 minutes.

8) Remove the supernatant by pipetting carefully without disturbing the pellet. If the pellet is disturbed, centrifuge again before removing the supernatant.

9) Add 500 µl of Washing Solution to each tube.

10) Vortex and re-suspend the pellet in the Washing Solution.

11) Centrifuge at 10,000 rpm for 3 minutes.

12) Remove the supernatant by pipetting carefully without disturbing the pellet. If the pellet is disturbed, centrifuge again before removing the supernatant.

13) Add 250 µl of Extraction Buffer to each tube.

14) Vortex and completely dissolve the pellet.

15) Transfer 200 µl from each tube to a 96-well plate.

16) Read the OD at 510-550 nm.



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## CALCULATION OF COLLAGEN CONTENT IN SAMPLES

1. Average the duplicate OD values for the standards, blanks (B), and test samples.

2. Subtract the blank (B) values from the averaged OD values of the standards and test samples.

3. Plot the OD values of the standards on the y-axis and the standard concentrations (μg/ml) on the x-axis. Figure 1 shows an example of a standard curve for this assay.

4. The collagen concentration (μg/ml) in test samples can be calculated using regression analysis. Multiply by the sample dilution factor to obtain the collagen concentration in the original sample specimens.

5. If the OD values of your samples are outside the standard curve range, then further dilution or concentration of your samples will be necessary.

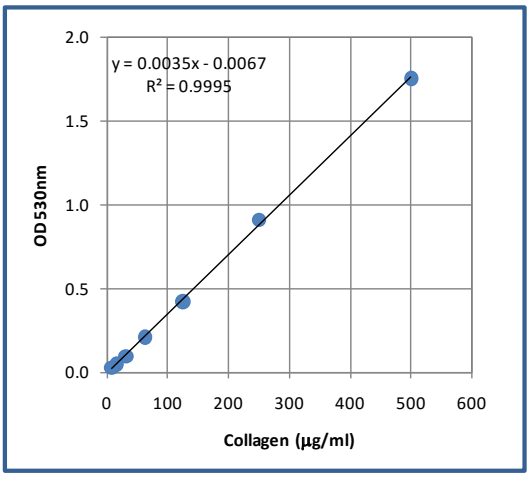


Figure 1. Typical Standard Curve

## CULTURE MEDIA

Samples containing higher concentrations of serum can cause high background values. Therefore, we recommend reducing the serum supplement concentration or diluting the cell culture media down to 5% using PBS.

## SAMPLE CONCENTRATION

The concentration of collagen in culture media is generally low, therefore it is difficult to detect collagen within the standard range of this kit. We recommend a sample concentration process using our Concentrating Solution (Catalog # 90626). Furthermore, a negative control using culture media should be used since this concentration method may result in elevated background levels.

1) Take 1 ml culture medium.

2) Add 250 μl of Concentrating Solution.

3) Vortex and incubate at 4°C for 16-24 hours.

4) Centrifuge at 10,000 rpm for 3 minutes.

5) Discard supernatant.

6) Add 100 μl of 0.05M acetic acid to dissolve the pellet. Use this solution as your sample.

7) Calculated collagen concentration will be multiplied by 0.1 as the dilution factor.



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