Sirius Red Total Collagen Detection Assay Kit

Catalog # 9062

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	Assay kit to quantify total collagen content in samples
FORMAT:	96-well ELISA plate with removeable strips
ASSAY TYPE:	Colorimetric assay
ASSAY TIME:	30 minutes
STANDARD RANGE:	500 µg/ml to 8 µg/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Tissue homogenate, cell culture medium, and cultured cells
RECOMMENDED SAMPLE DILUTIONS:	Varies depending on sample type
CHROMOGEN:	N/A (read at 510-550 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	N/A
NOTES:	This product is NOT the Sirius Red Total Collagen Detection Assay PLATE Kit (Cat # 9062P)

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16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com

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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. Chondrex, Inc. provides a Sirius Red Total Collagen Detection Assay Kit for detecting the total collagen content in 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, Chondrex, Inc. recommends our Type I Collagen Detection ELISA kits and Type II Collagen Detection ELISA Kit. For convenience, the same sample preparations may be used for both the Collagen Detection ELISA Kits and the Sirius Red Total Collagen Detection Assay Kit. For more information, please visit www.chondrex.com or contact support@chondrex.com.

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 (Cat # 90626) for cell culture media samples is NOT included. Please contact Chondrex, Inc. customer service support@chondrex.com to place an order.

PREPARING SAMPLES

Tissue specimens and cultured cells can be used; however, solid samples must be solubilized for this assay. Culture media samples may require a concentration process (please see protocol). In addition, 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)

Chondrex, Inc. recommend our “Tips for Collagen Solubilization” to prepare pepsin-soluble collagen samples. Please contact Chondrex, Inc. customer service support@chondrex.com for more information.

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CULTURE MEDIA

Samples containing higher concentrations of serum can cause high background values. Therefore, Chondrex, Inc. recommends reducing the serum supplement concentration in cell culture media down to 5% using PBS.

CONCENTRATING SAMPLES

The concentration of collagen in culture media is generally low, therefore it is difficult to detect collagen in culture media samples within the standard range of this kit. Chondrex, Inc. recommends a sample concentration process using our Concentrating Solution (Cat # 90626). Furthermore, a negative control using culture media should be used as 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 should be multiplied by a 0.1 dilution factor.

ASSAY OUTLINE

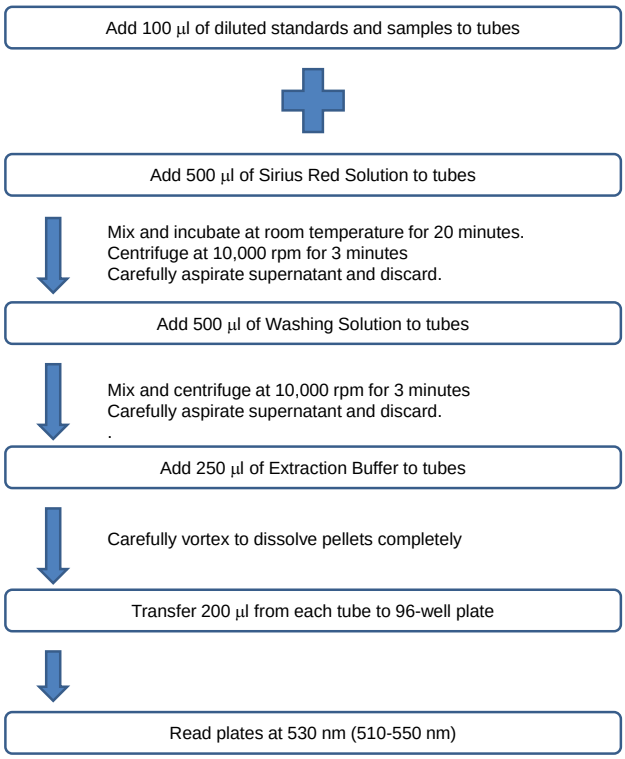

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PLATE MAPPING

	1	2	3	4	5	6	7	8	9	10	11	12
A	500	500	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	250	250	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	125	125	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	63	63	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	31	31	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	16	16	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	8	8	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
H	B	B	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40

Standards Samples

NOTES BEFORE USING ASSAY

NOTE 1: It is recommended that the standard and samples be run in duplicate.

NOTE 2: Warm up all buffers to room temperature before use.

NOTE 3: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

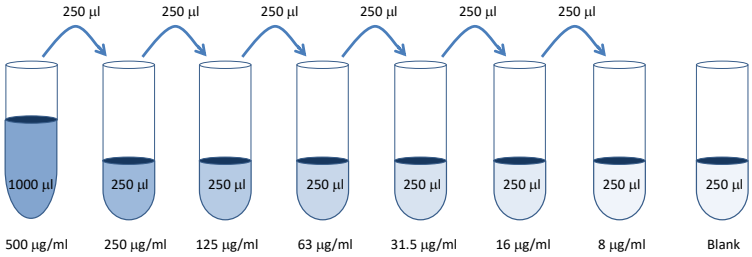
NOTE 4: Partially used stock reagents may be kept in their original vials and stored at -20°C for use in a future assay.

NOTE 5: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

ASSAY PROCEDURE

1. **Prepare 1X Acetic Acid**: Using purified distilled water, prepare enough 1X acetic acid (0.05M) solution for the standards and samples.

2. **Prepare Standard Solutions**: 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 process five times to make 125, 63, 31.5, 16, and 8 µg/ml solutions.



3. **Prepare Samples**: Prepare sample solutions in 1.5 ml centrifuge tubes or disposable culture tubes. If the collagen concentrations of the samples are unknown, preparing multiple samples with varying dilutions using 0.05M acetic acid is recommended.

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

5. **Add Sirius Red Solution**: Add 500 µl of Sirius Red Solution to each tube. Vortex and incubate for 20 minutes at room temperature.

6. **Centrifuge**: Centrifuge at 10,000 rpm for 3 minutes. Remove the supernatant by pipetting carefully without disturbing the pellet. If the pellet is disturbed, centrifuge again before removing the supernatant.

7. **Add Washing Solution**: Add 500 µl of Washing Solution to each tube. Vortex and re-suspend the pellet in the Washing Solution.

8. **Centrifuge**: Centrifuge at 10,000 rpm for 3 minutes. Remove the supernatant by pipetting carefully without disturbing the pellet. If the pellet is disturbed, centrifuge again before removing the supernatant.

9. **Add Extraction Solution**: Add 250 µl of Extraction Buffer to each tube. Vortex and completely dissolve the pellet.

10. **Read**: Transfer 200 µl from each tube to a 96-well plate. Read the OD at 510-550 nm.

CALCULATING RESULTS

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

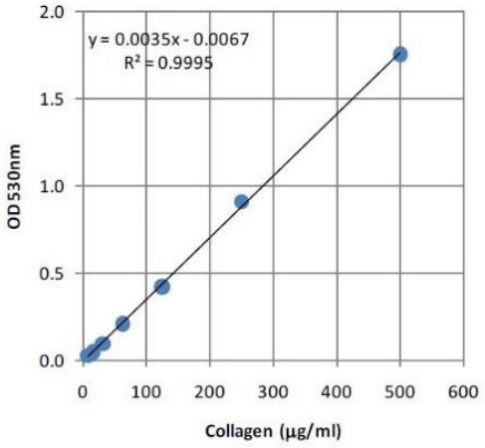
2. Subtract the blank (B) values from the averaged OD values of the standards and test samples.

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 the samples are outside the standard curve range, then it will be necessary to further dilute or concentrate samples.

Figure 1 - A Typical Standard Curve for the Sirius Red Total Detection Assay Kit



TROUBLESHOOTING

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

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