Sirius Red Total Collagen Detection Assay Plate Kit

Catalog # 9062P

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	Assay kit to quantify total collagen levels
FORMAT:	96-well ELISA plate with removeable strips
ASSAY TYPE:	Colorimetric assay
ASSAY TIME:	< 1 hour
STANDARD RANGE:	500 - 8 µg/ml
NUMBER OF SAMPLES:	Up to 80 (duplicate) samples/kit
SAMPLE TYPES:	Solubilized tissue, cell culture media, and cultured cells
RECOMMENDED SAMPLE DILUTIONS:	Varies depending on sample type
CHROMOGEN:	N/A
STORAGE:	-20°C
VALIDATION DATA:	N/A
NOTES:	This product is NOT the Sirius Red Total Collagen Detection Assay Kit (Cat # 9062)

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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. Therefore, Chondrex, Inc. provides a Sirius Red Collagen Detection Assay Plate Kit designed to detect the total collagen content in various samples. This kit uses an ELISA plate for various collagen-containing samples such as tissue specimens, cell culture media, and cultured cells. This protocol is ideal for users who have many samples because the total assay working time is less than 60 minutes and 80 samples can be measured in duplicate. Due to the low level of collagen in cell culture media, additional concentration steps may be necessary. Concentrating Solution (Cat # 90626) for cell culture media samples is NOT included in this kit. Please contact customer service support@chondrex.com to place an order.

For determining levels of collagen from individual species or different types of samples, Chondrex, Inc. recommends the Sirius Red Collagen Detection Assay Kit using the tube assay method (Cat # 9062), Type I Collagen Detection ELISA Kits, and the Type II Collagen Detection ELISA Kit. Please visit www.chondrex.com for more information. For convenience, the same sample preparation methods may be used for both the Collagen Detection ELISA Kits and the Sirius Red Collagen Detection Assay Kits.

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/*
Flat-bottom 96-Well ELISA Plate (9026)	2 each	8-well strips x 12	-20°C/*
V-bottom 96-Well Plate for Centrifugation (90261)	2 each	2 plates	-20°C/*
Flexible 96-Well Plate for Sample Dilution (90262)	1 each	1 plate	-20°C/*
96-Well ELISA Plate Sealer (90263)	2 each	2 each	-20°C/*

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

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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 concentrating process. In addition, heat-denatured collagen tends to have a lower binding affinity for Sirius Red, resulting in underestimated values. Chondrex, Inc. provides "Tips for Collagen Solubilization" to prepare pepsin-soluble collagen samples. Please contact customer service support@chondrex.com for more information. 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)

CULTURE MEDIA

Samples containing higher concentrations of serum can cause high background values. Therefore, Chondrex, Inc. recommends reducing the serum supplement concentration in samples such as cell culture media to lower than 5% using 0.05M PBS pH 7.4.

SAMPLE CONCENTRATION

The concentration of collagen in culture media is generally low, making it difficult to detect collagen within the standard range of this kit. Chondrex, Inc. recommends a sample concentration process using 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 sample.
7. Calculated collagen concentration should be multiplied by a 0.1 dilution factor.

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.

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PLATE LAYOUT

	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

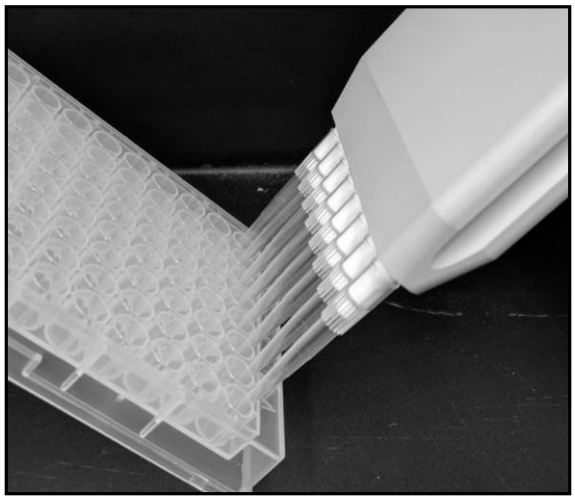

Figure 1 - Removing Supernatant by Pipetting

ASSAY OUTLINE

Add 50 µl of diluted standards and samples to wells of V-bottom 96-well plate

Add 250 µl of Sirius Red Solution to wells of V-bottom 96-well plate

Mix and incubate at room temperature for 20 minutes.
Centrifuge at 3,000 rpm for 10 minutes
Carefully aspirate 250 µl of supernatant and discard.

Add 250 µl of Washing Solution to wells of V-bottom 96-well plate

Mix and centrifuge at 3,000 rpm for 10 minutes
Carefully aspirate 250 µl of supernatant and discard.
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Add 150 µl of Extraction Buffer to wells of V-bottom 96-well plate

Carefully pipet to dissolve pellets completely

Transfer 150 µl from each well to a new flat-bottom 96-well plate

Read plates at 530 nm (510-550 nm)

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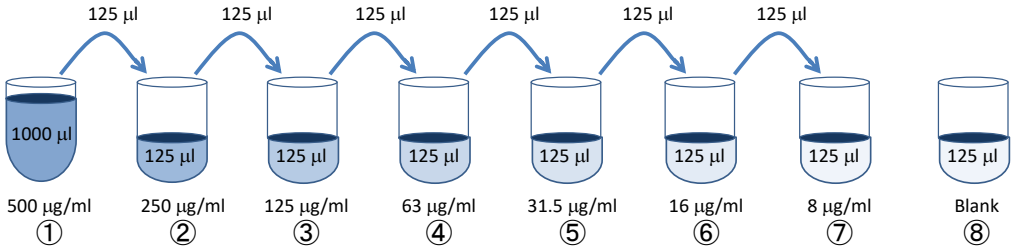
ASSAY PROCEDURE

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

2. **Prepare Standard Solutions**: using a flexible 96-well plate, centrifuge tubes, or disposable culture tubes, add 125 µl of 0.05M acetic acid to seven blank spaces. Mix 125 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 standard solutions.



3. **Prepare Sample Solutions**: prepare samples using flexible 96-well plates, small centrifuge tubes, or disposable culture tubes. Because the collagen concentration of samples is unknown, preparing multiple samples with various dilutions using 0.05M acetic acid is recommended.

4. **Add Standards and Samples**: add 50 µl of blank, diluted standard solutions, and samples to a V-bottom 96-well plate in duplicate.

5. **Add Sirius Red Solution:** add 250 µl of Sirius Red Solution to each well. Pipet several times to mix the standards and samples with the dye solution and then apply the plate sealer. Incubate for 20 minutes at room temperature.

6. **Centrifuge**: centrifuge at 3,000 rpm for 10 minutes.

7. **Discard Supernatant**: remove the plate sealer, tilt the plate at a 45-degree angle (Figure 1), and aspirate 250 µl of supernatant without disturbing the pellet. If the pellet is disturbed, centrifuge again before removing the supernatant. Discard supernatant.

NOTE: Figure 2 shows typical pellets in the standard wells after removing supernatants

Figure 2 - Typical Pellets in the Wells

Figure 3 - Typical Pellets in the Wells

Figure 4 - Typical Color in the Wells

8. **Add Washing Solution**: add 250 µl of Washing Solution to each well and re-apply the plate sealer.

9. **Centrifuge**: centrifuge at 3,000 rpm for 10 minutes.

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10. **Discard Supernatant**: remove the plate sealer, tilt the plate at a 45-degree angle (Figure 1), and aspirate 250 µl of supernatant without disturbing the pellet. If the pellet is disturbed, centrifuge again before removing the supernatant. Discard supernatant.

    NOTE: Figure 3 shows typical pellets in the standard wells after removing supernatant.

11. **Add Extraction Buffer:** add 150 µl of Extraction Buffer to each well. Pipet several times to dissolve the pellet completely.

    NOTE: Figure 4 shows typical color in the standard wells after dissolving the pellet.

12. **Transfer**: transfer 150 µl from each well to a clean, flat-bottom 96-well plate.

13. **Read Plate**: read the plate at 530 nm (510-550 nm).

CALCULATING RESULTS

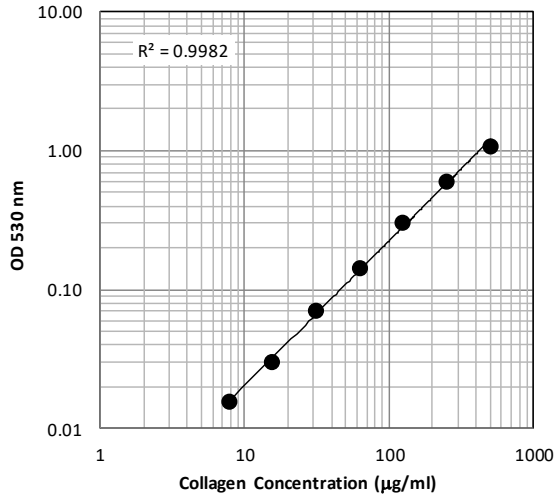
1. Average the duplicate OD values for the blank, standards, and test samples.

2. Subtract the "blank" (B) values from the averaged OD values in step 1.

3. Plot the OD values of standards against the concentration of the standard (µg/ml). Using a log/log plot will linearize the data. Figure 5 shows a representative experiment where the standard range is 8 - 500 µg/ml.

4. The µg/ml of collagen 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. If the OD values of the samples are outside of the standard curve range, the re-assay the samples at different dilutions.

Figure 5 - A Typical Standard Curve for the Sirius Red Total Collagen Detection Assay Plate Kit



TROUBLESHOOTING

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

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