Version 1d Last updated 31 October 2019

ab242291 Soluble Collagen Assay Kit

View Soluble Collagen Assay Kit datasheet: www.abcam.com/ab242291

For the detection of soluble collagen from cell or tissue sample.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

The Soluble Collagen Assay Kit (ab242291) provides a convenient colorimetric method for the detection of soluble collagen from cell or tissue samples. First, the unknown samples or collagen standards are added to a 96 well plate and dried down overnight. Then, a Sirius Red reagent is added to stain the [Gly-x-y] triple helix structure of collagen. Finally, the stained collagen is washed with an Acidic Reagent, eluted from the plate with a Basic Reagent, transferred to a new 96 well plate and measured by a plate spectrophotometer.

The amount of collagen in the unknown samples is determined by comparing with a predetermined collagen standard curve. The provided reagents are sufficient for the evaluation of 96 assays including standards and unknown samples.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed.

Add 100 µL of collagen standards or unkown samples into a 96-well plate. Evaporate to dryness overnight at 37°C.

Wash the wells, and remove excess water, add 150 µL of the Sirius Re Reagent to each well. Incubate for 60 minutes at RT on an orbital shaker.

Wash the wells 4 times with 200 µL of 5% Acetic Acid solution.

Add 150 µL of Extraction Reagent and incubate for 30 mins at RT on an orbital shaker.

Read absorbance immediately on a microplate reader using 540/560 nm.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at 4°C immediately upon receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.
- Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage condition
Collagen Standard	500 µL	4°C
Sirius Red Reagent	15 mL	RT
Extraction Reagent	15 mL	4°C
10X PBS	10 mL	4°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 96 well ELISA strips or 96 well microtiter plate
- 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Microplate reader capable of reading at 540-560 nm
- Pepsin
- 2.5% and 5% (v/v) Acetic Acid
- 2N Sodium hydroxide solution

6. Reagent Preparation

– All regents in this kit are ready to use as supplied.

7. Standard Preparation

– Always prepare a fresh set of standards for every use.
– Discard working standard dilutions after use as they do not store well.

7.1 Prepare dilution series of Collagen standards in the concentration range of 0 to 500 µg/mL by diluting the Collagen Standard in cold PBS, shown in the table below:

Standard #	3 mg/mL Collagen Standard (µL)	Cold PBS (µL)	Collagen (µg/mL)
1	100	500	500
2	250 of tube #1	250	250
3	250 of tube #2	250	125
4	250 of tube #2	250	62.5
5	250 of tube #4	250	31.2
6	250 of tube #5	250	15.6
7	250 of tube #6	250	7.8
8	0	250	0

8. Sample Preparation

– The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

ΔNote: This kit is not recommended for serum, plasma, or urine samples

8.1 Cells:

– Resuspend 1-2 x 10^7 cells in 1 mL of 2.5% Acetic Acid containing 0.1 mg/mL Pepsin. Disrupt cells on ice by dounce homogenization.
– Sonicate the homogenate on ice with a probe sonicator. Centrifuge the homogenate at 12,000 x g for 10 minutes.
– Recover the supernatant and transfer to a new tube. Determine the protein concentration by protein assay. Neutralize the pH of the sample in two steps. First add 2N Sodium Hyrdoxide solution 1:6 directly into the sample.
– Then add 10X PBS 1:10 directly to the sample to a final concentration of 1X PBS (for example, add 100 µL of 2N Sodium Hydroxide solution to 500 µL of sample, and then add 67 µL of 10X PBS to the sample). Store unused final sample at -80°C.

8.2 Tissue:

– Homogenize 100 mg of tissue in 1 mL of 2.5% Acetic Acid containing 0.1 mg/mL Pepsin.
– Disrupt tissue on ice by dounce homogenization. Sonicate the homogenate on ice with a probe sonicator. Centrifuge the homogenate at 12,000 x g for 10 minutes.
– Recover the supernatant and transfer to a new tube. Determine the protein concentration by protein assay. Neutralize the pH of the sample in two steps. F
– First add 2N Sodium Hydroxide solution 1:6 directly into the sample. Then add 10X PBS 1:10 directly to the sample to a final concentration of 1X PBS (for example, add 100 µL of 2N Sodium Hydroxide solution to 500 µL of sample, and then add 67 µL of 10X PBS to the sample). Store unused final sample at -80°C.

9. Assay Procedure

9.1 Prepare and mix all reagents thoroughly before use. Each sample, unknown and standard should be assayed in duplicate.

9.2 Add 100 µL of collagen standards or unknown samples to a 96 well microplate.

9.3 Evaporate to dryness overnight in a 37°C oven for 16 hours.

9.4 Wash the wells 3 times with 200 µL of distilled water with aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess water.

9.5 Add 150 µL of the Sirius Red Reagent to each well.

9.6 Incubate for 60 minutes at room temperature on an orbital shaker.

9.7 Wash the wells 4 times with 200 µL of 5% Acetic Acid with aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Acidic Reagent.

9.8 Add 150 µL of Extraction Reagent to each well.

9.9 Incubate 30 minutes at room temperature on an orbital shaker.

9.10 Transfer 100 µL to the wells of a new plate.

9.11 Read absorbance of each well on a microplate reader using 540-560 nm as the primary wavelength.

10. Typical Data

The following figure demonstrates typical blot results for the CML-BSA Immunoblot Control. One should use the data below for reference only. This data should not be used to interpret actual results.

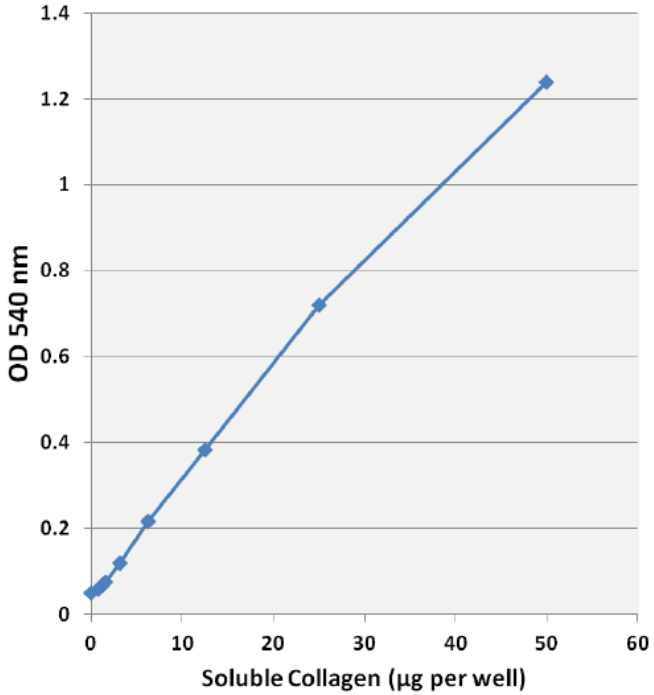

Figure 1. Soluble Collagen Standard Curve.

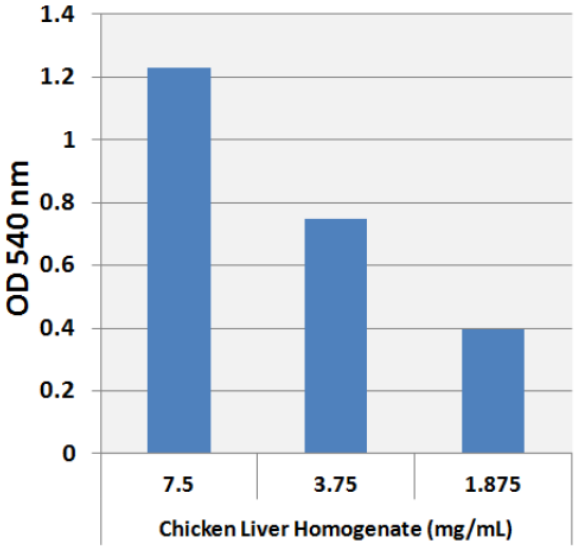

Figure 2. Detection of Soluble Collagen in Chicken Liver. Chicken liver was homogenized in 2.5% Acetic Acid containing 0.1 mg/mL Pepsin, pH neutralized, and diluted into PBS according to the Preparation of Samples Section. Samples were tested according to the Assay Protocol.

11. Notes

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