Product Manual

Soluble Collagen Assay Kit

Catalog Number

MET-5016 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Collagen serves as the major structural component of animal connective tissues. Collagen is found in fibrous tissues such as skin and ligaments, but is also found in large amounts in bone, cartilage, and cornea. When hydrolyzed, collagen forms gelatin which is used in the food industry as well as medically to treat bone and skin disorders. Collagen makes up about 30% of the total protein content in animals, making it the most abundant animal protein. Hydroxyproline, a modified form of proline, is found almost exclusively in the protein collagen, in the Y position of the repeating tripeptide Gly-X-Y. By allowing sharp twisting of the collagen helix, hydroxyproline helps to stabilize the structure of collagen.

Collagen plays an important structural role in the heart. The cardiac extracellular matrix, which is made up mostly of fibrillar collagen, preserves myocardial integrity, allows for heart force transmission, and contributes to myocyte orientation. Any interruption of the finely balanced regulation of collagen synthesis, post-synthetic assembly, post-translational modification and degradation may have large effects on myocardial function. In addition, collagen has several clinical uses. Collagen has been used extensively in cosmetic surgery to aid in the healing process for burn patients. Collagen has also been used to reconstruct bone in dental, orthopedic, and other surgical scenarios. Collagen is used for bone grafts because of its triple helical structure which makes it a very strong molecule. It is also optimal for use in bones because it does not compromise the structural integrity of the skeleton. Finally, both human and bovine collagen has been used as dermal fillers to treat wrinkling and skin aging.

Cell Biolabs' Soluble Collagen Assay Kit 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.

Related Products

1. STA-675: Hydroxyproline Assay Kit (Total Collagen Assay)

Kit Components

1. Collagen Standard (Part No. 50161B): One 500 µL vial containing 3 mg/mL Type I Collagen.
2. Sirius Red Reagent (Part No. 50162A): One 15 mL bottle.
3. Extraction Solution (Part No. 50164A): One 15 mL bottle.
4. 10X PBS (Part No. 50166A): One 10 mL bottle.

Materials Not Supplied

1. Distilled water
2. Phosphate Buffered Saline (PBS)
3. 96 well ELISA strips or 96 well microtiter plate

4. Pepsin
5. 2.5% and 5% (v/v) Acetic Acid
6. 2 N NaOH

Storage

Upon receipt, store the Sirius Red Reagent at room temperature and store the rest of the kit at 4ºC.

Preparation of Standard Curve

Prepare a dilution series of Collagen standards in the concentration range of 0 to 500 µg/mL by diluting the Collagen Standard in cold PBS (Table 1).

Standard Tubes	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 #3	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

Table 1. Preparation of Collagen Standards.

Preparation of Samples

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

- Cells: Resuspend 1-2 x 10^7 cells in 1 mL of 2.5% Acetic Acid containing 0.1 mg/mL Pepsin according to ref. 6 (see p. 7). 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 NaOH 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 NaOH solution to 500 µL of sample, and then add 67 µL of 10X PBS to the sample). Store unused final sample at -80°C.
- Tissue: Homogenize 100 mg of tissue in 1 mL of 2.5% Acetic Acid containing 0.1 mg/mL Pepsin according to ref. 6 (see p. 6). 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. First add 2N NaOH 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 NaOH solution to 500 µL of sample, and then add 67 µL of 10X PBS to the sample). Store unused final sample at -80°C.

  Note: This kit is <u>not</u> recommended for serum, plasma, or urine samples. Perform acid hydrolysis and measure the collagen marker hydroxyproline using Cell Biolabs' Hydroxyproline Assay Kit (STA-675).

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, unknown and standard should be assayed in duplicate.
2. Add 100 µL of collagen standards or unknown samples to a 96 well microplate.
3. Evaporate to dryness overnight in a 37ºC oven for 16 hours.
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.
5. Add 150 µL of the Sirius Red Reagent to each well.
6. Incubate for 60 minutes at room temperature on an orbital shaker.
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.
8. Add 150 µL of Extraction Solution to each well.
9. Incubate 30 minutes at room temperature on an orbital shaker.
10. Transfer 100 µL to the wells of a new plate.
11. Read absorbance of each well on a microplate reader using 540-560 nm as the primary wavelength.

Example of Results

The following figures demonstrate typical Soluble Collagen Assay Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

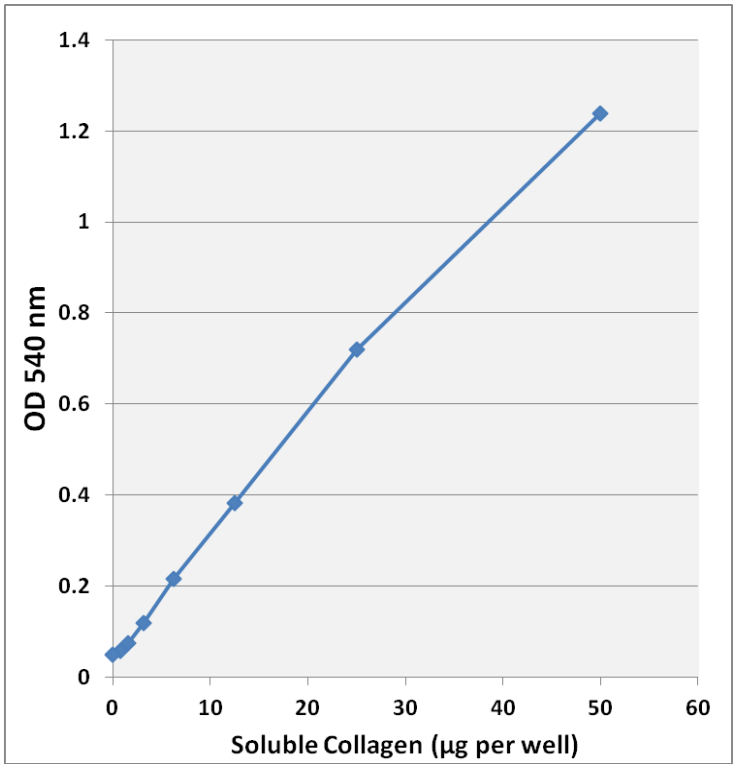

Figure 2: Soluble Collagen Standard Curve.

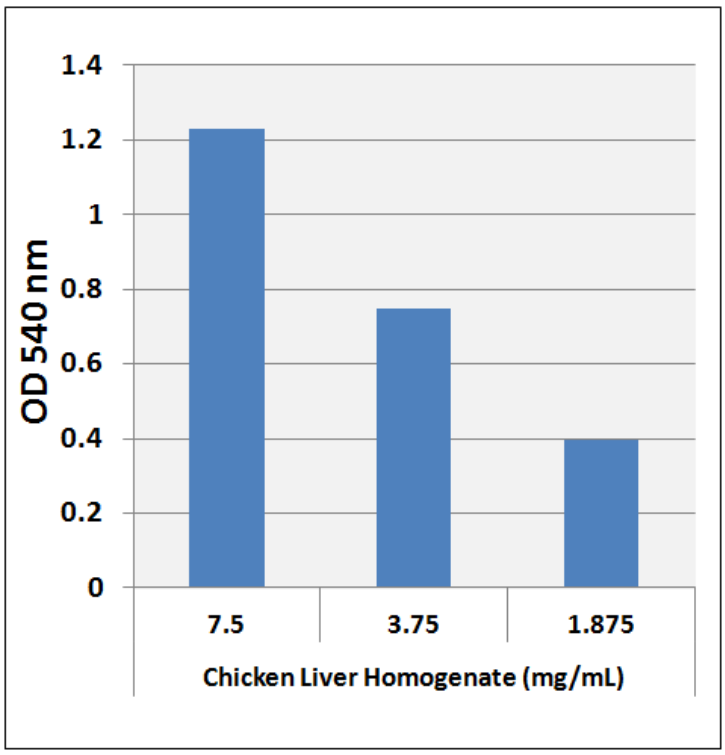

Figure 3: Detection of Soluble Collagen in Chicken Liver. Chicken liver was homogenized in 2.5% Acetic Acid containing 0.1 mg/mL Pepsin, pH neutralized, and diluted into PBS according to the Preparation of Samples Section. Samples were tested according to the Assay Protocol.

References

1. Di Lullo GA.; Sweeney SM.; Körkkö J; Ala-Kokko L and San Antonio JD. (2002). *J. Biol. Chem*. **277**: 4223–4231
2. Gorres K.L and Raines RT. (2010) *Crit Rev Biochem Mol Biol.* 4**5**: 106-124
3. Cunniffe, G and O'Brien F (2011). *J.Minerals Metals and Mater. Soc*. **63**: 66–73
4. Oliveira S, Ringshia R, Legeros R, Clark E, Terracio L, Teixeira C, and Yost M (2009). *J. Biomed. Mater*. **94**: 371–379.
5. Horn MA and Trafford AW (2015). *J Mol. Cell Cardiol*. Epub ahead of print.
6. Chan D., Lamande R, Cole WG, and Bateman JF (1990). *Biochem J*. **269**: 175-181.

Recent Product Citations

1. Alqahtani, M.S. et al. (2020). Wound-healing potential of curcumin loaded lignin nanoparticles. *J Drug Deliv Sci Technol*. doi: 10.1016/j.jddst.2020.102020.
2. Sears, V. et al. (2020). Harnessing mesenchymal stem cell secretome: effect of extracellular matrices on pro-angiogenic signaling. *Biotechnol Bioeng*. doi: 10.1002/bit.27272.
3. MacKnight, H.P. et al. (2019). The interaction of ceramide 1-phosphate with group IVA cytosolic phospholipase A2 coordinates acute wound healing and repair. *Sci Signal*. **12**(610). pii: eaav5918. doi: 10.1126/scisignal. aav5918.
4. Imai, J. et al. (2019). Flagellin-mediated activation of IL-33-ST2 signaling by a pathobiont promotes intestinal fibrosis. *Mucosal Immunol*. **12**(3):632-643. doi: 10.1038/s41385-019-0138-4.

Warranty

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Contact Information

Cell Biolabs, Inc.
5628 Copley Drive
San Diego, CA 92111
Worldwide: +1 858 271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com