Total CollagenMicroplate Assay Kit

User Manual

Catalog # CAK1276

(Version 1.1A)

Detection and Quantification of Total CollagenContentUrine, Serum, Plasma, Tissue extracts and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

COLLAGEN is the key structural protein of connective tissue and the most abundant protein in mammals. It occurs in many different types and forms with Types I -V being the most common. Aside from the crucial role it plays in the body, it has numerous medical applications such as its use in reconstructive surgery including bone and skin grafts. It is also commonly used in cosmetics due to its anti-aging and skin healing properties.

Total Collagen Microplate Assay Kit is a simple and sensitive assay to detect small amounts of collagens in a variety of samples. The assay is based on the acid hydrolysis of samples to form hydrolysates and Hydroxyproline. This released Hydroxyproline gets oxidized to form a reaction intermediate, which further in the reaction, forms a chromophore can be measured at a colorimetric readout at 560 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Substrate	Powderx 1	4 °C, keep in dark
Substrate Diluent	8 mlx 1	4 °C, keep in dark
Stop Solution	4 ml x 1	4 °C
Dye Reagent	Powderx 1	4 °C, keep in dark
Dye Reagent Diluent	4 mlx 1	4 °C
Standard	Powderx 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate:add 8 ml Substrate Diluentto dissolve before use.

Dye Reagent: add 4 ml Dye Reagent Diluentto dissolve before use.

Standard:add 0.25 mldistilled water to dissolve before use, and add 0.25 ml of 12 M concentrated HCl (not provided).Securely tighten cap and hydrolyze at 120°C for 3 hours. Cool vial on ice, then spin down the vial contents,then add 10 mol/L NaOH adjust to pH 7.0. Make up to a total volume of 1ml with distilled water, the concentration will be 1 mg/ml.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 560 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Concentrated HCl
8. 10 mol/L NaOH
9. Autoclaves Sterilizer

IV. SAMPLE PREPARATION

1.For tissue samples

Weighout 0.1g tissue in the glass tube, homogenize in 1 mldistilled water. To a 100 µl of sample homogenate, add 100 µl concentrated HCl (not provided) in a pressure-tight polypropylene screw-capped vial,put it into autoclaves sterilizer, hydrolyze samples at 120°C for 3 hours.Vortex and centrifuge at 10000 x g for 5 minutes to remove precipitate, then add 10 mol/L NaOH adjust to pH 7.0.Make up to a total volume of 400 µl with distilled water.

2. For urine, serum, plasma and other liquid samples

Hydrolyze samples with equal volumes of concentrated HCl (1:1, not provided) in a pressure-tight polypropylene screw-capped vial,put it into autoclaves sterilizer, hydrolyze samples at 120°C for 3 hours. Vortex and centrifuge at 10000 x g for 5 minutes to remove precipitate, then add 10 mol/L NaOH adjust to pH 7.0.Make up to a total volume of 400 µl with distilled water.

V. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent	Sample	Standard	Blank
Sample	40 µl	--	--
Standard	--	40 µl	--
Distilled water	--	--	40 µl
Substrate	80 µl	80 µl	80 µl
Mix, stand at room temperature for 20 minutes.			
Stop Solution	40 µl	40 µl	40 µl
Mix, stand at room temperature for 10 minutes.			
Dye Reagent	40 µl	40 µl	40 µl
Mix,put it in the oven,65°Cfor 20 minutes,cool to room temperature, measured at 560 nm and record the absorbance.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the weight of sample

$$\text{Collagen (mg/g)} = C_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) \times V_{Standard} / V_{Sample} / (W / V_{Assay}) \times n \times 10$$

$$= 40 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

2. According to the volume of sample

$$\text{Collagen (mg/ml)} = C_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) \times V_{Standard} / V_{Sample} \times n$$

$$= 4 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 1 mg/ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

$V_{Standard}$: the volume of sample, 0.04 ml;

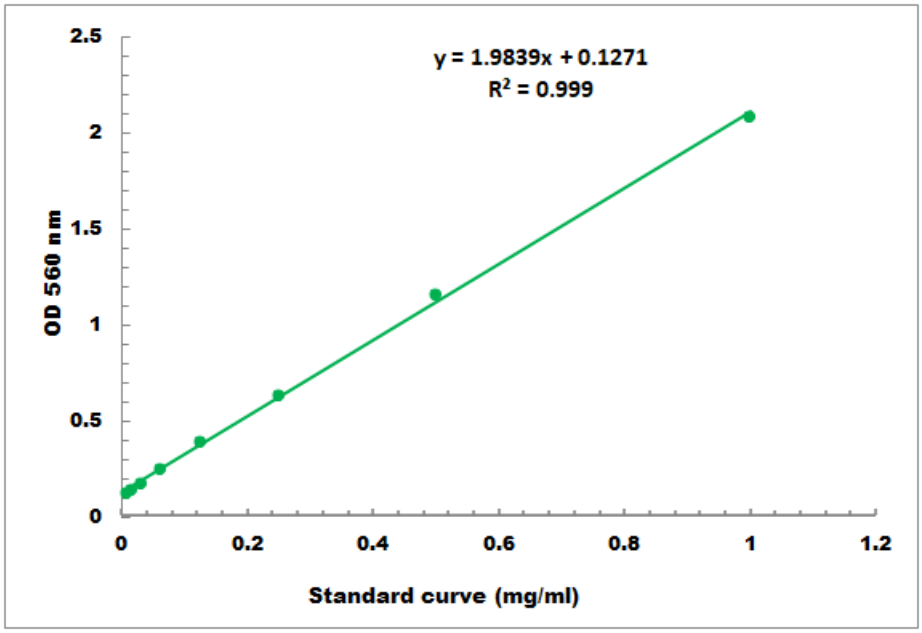
V_{Sample}: the volume of sample, 0.04 ml;

V_{Assay}: thetotal volume of distilled waterin sample preparation, 1 ml;

n: dilution factor, n=4.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.01mg/ml-1 mg/ml

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES