Total Collagen

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1276

(Version 1.2A)

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 Colorimetric 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	

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. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.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. Store at 4 °C for 2 days or -20°C for 2-3 months. Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be1/0.5/0.25/0.125/0.0625/ 0.0312/0.0156mg/ml.

Substrate: Add 8 ml Substrate Diluentto dissolve before use. Keep in dark and store at 4 °C for 1 week or -20°C for 1-3 monthsafter reconstitution.

Dye Reagent: Add 4 ml Dye Reagent Diluentto dissolve before use. Keep in dark for immediateuse or store at 4 °C for 48 hours after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. 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:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Sample}-OD_{Blank})-\text{Intercept}}{\text{Slope}}\times n(mg/ml)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the weight of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times W\times(V_{Sample}/V_{Assay})}(mg/g)$$

2.2According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}}(mg/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

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

$V_{Standard}$: the volume of standard in assay procedure, µl

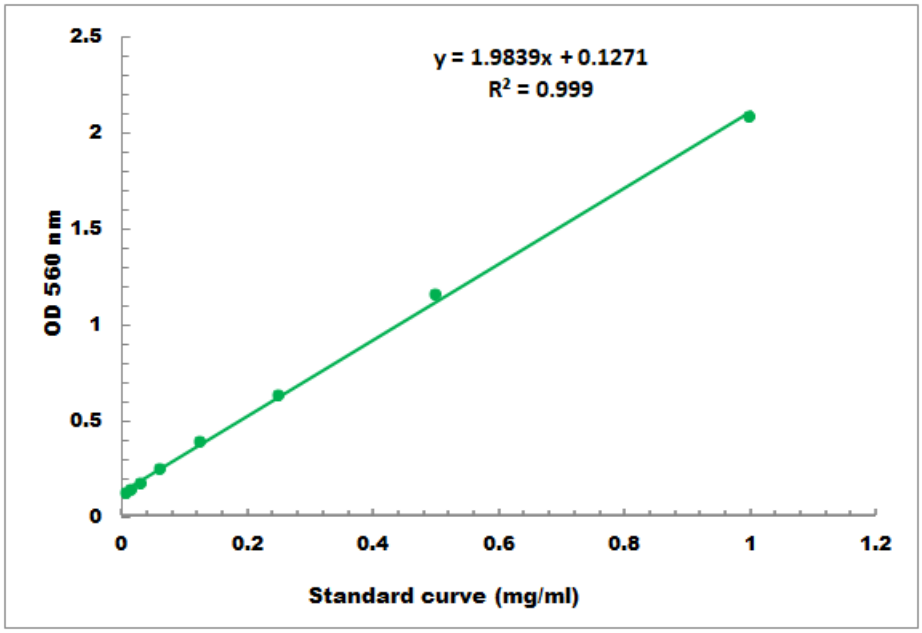
V_{Sample}: the volume of sample in assay procedure, µl

V_{Assay}: the volume of Assay Buffer, µl

W: the weight of sample, g

VIII. 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