Hydroxyproline

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1067

(Version 1.5D)

Detection and Quantification of Hydroxyproline(HYP)ContentUrine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

I. INTRODUCTION

Hydroxyproline (4-hydroxyproline) is a common non-proteinogenic amino acid. It is found only in collagen and elastin in mammals but exists in a number of other proteins in plants. Hydroxyproline is formed only as a post-translational modification in the peptide chain and proline hydroxylase does not hydroxylate free proline. Hydroxyproline in tissue hydrolysates is a direct measure of the amount of collagen or gelatin present. A variety of disease states are believed to affect collagen turnover and can cause elevated serum or urine hydroxyproline. Such conditions range from neoplastic, inflammatory, renal or bone disease to endocrine and autoimmune disorders.

Hydroxyproline Colorimetric Microplate Assay Kit is a simple and sensitive assay to detect small amounts of hydroxyproline in a variety of samples.HYPmay react with Chloramine T and DMAB. The products 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. 6 mol/LHCl
8. 10 mol/L NaOH
9. Alcohol
10. Autoclaves Sterilizer

IV. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1 ml distilled watertogenerate 100mmol/L of standardstock solution, store at 4 °C for 2-3 monthsafter reconstitution.Then dilute to1mmol/L standard solutionby adding10 µl stock solution into 990µl distilled water.Then dilute to 200 µmol/L standard top solutionby adding 200 µl 1mmol/L standard solution into 800µl distilled water. Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be200/100/50/25/12.5/6.25/3.12µmol/L.

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 monthafter reconstitution.

Dye Reagent: Add 4 ml Dye Reagent Diluentto dissolve before use.Prepare fresh for immediate use.

V. SAMPLE PREPARATION

1.For cell samples

Collect cellinto centrifuge tube, discard the supernatant after centrifugation, add 1ml6mol/L HClfor 5 ×106 cell or bacteria, put it in oven of 110 °Cfor 6-12 hours orautoclaves sterilizer or 121 °C for 6-8 hours; then add 10 mol/L NaOHadjustto pH 7.0.

2.For tissue samples

Weighout 0.1g tissue in the glass tube, add1ml6mol/L HCl, put it in oven of 110 °Cfor 12-24 hours orautoclaves sterilizer or 6-8 hours,centrifuged at 16000g 25°C for 20minutes, take the supernatant into a new centrifuge tube, then add10 mol/L NaOHadjust to pH 7.0.

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

Add 0.9 ml alcohol for 0.1 ml liquid samples, mix; keep on ice for 10 minutes; centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

VI. 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

$$C=\frac{(OD_{Sample}-OD_{Blank})-\text{Intercept}}{\text{Slope}}\times n(\mu mol/L)$$

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 protein concentration of sample

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

2.2According to the quantity of cells

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times N\times(V_{Sample}/V_{Assay})}(\mu mol/10^{4})$$

2.3 According to the weight of sample

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

2.4According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}}\times 10(\mu mol/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, mmol/L = μmol/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

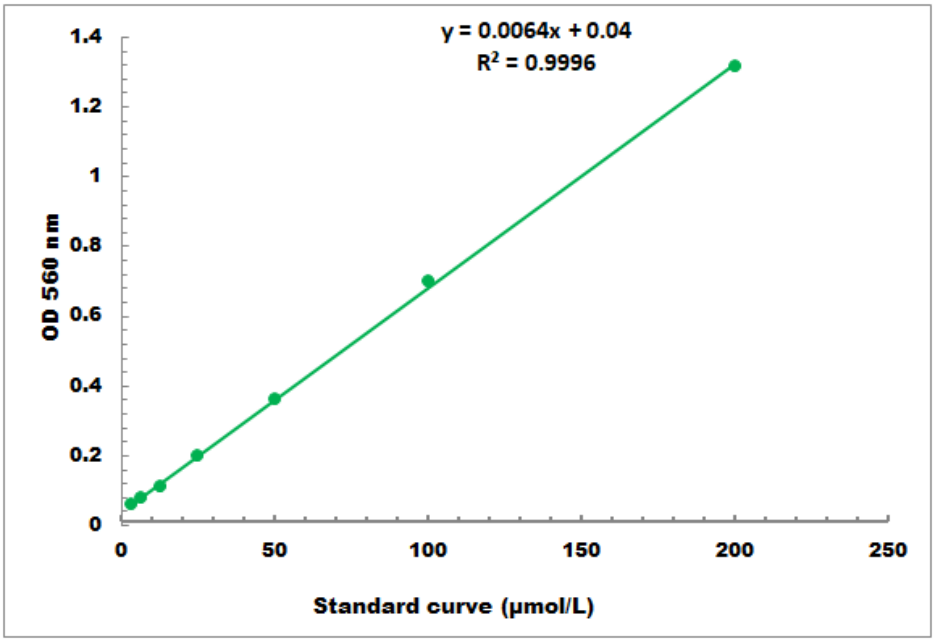
$C_{Protein}$: the sample protein concentration, mg/ml

W: the weight of sample, g

N: the quantity of cell, 10^4

VIII. TYPICAL DATA

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



Detection Range: 2µmol/L - 200 µmol/L