Hydroxyproline Microplate Assay Kit

User Manual

Catalog # CAK1067

(Version 1.4D)

Detection and Quantification of Hydroxyproline (HYP) Content Urine, 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 Microplate Assay Kit is a simple and sensitive assay to detect small amounts of hydroxyproline in a variety of samples. HYP may 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	Powder x 1	4 °C, keep in dark
Substrate Diluent	8 ml x 1	4 °C, keep in dark
Stop Solution	4 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C, keep in dark
Dye Reagent Diluent	4 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate: add 8 ml Substrate Diluent to dissolve before use.

Dye Reagent: add 4 ml Dye Reagent Diluent to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use; add 10 µl into 990 µl distilled water; then 200 µl into 800 µl distilled water, the concentration will be 200 µmol/L.

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/L HCl
8. 10 mol/L NaOH
9. Alcohol
10. Autoclaves Sterilizer

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml 10 mol/L NaOH for 5×10^6 cell or bacteria, put it into autoclaves sterilizer, 121 °C for 30 minutes; add 6mol/L HCl adjust to pH 7.0.

2. For tissue samples

Weigh out 0.1 g tissue in the glass tube, add 1 ml 6mol/L HCl, put it in oven of 110 °C for 6 to 12 hours, centrifuged at 16000g 25 °C for 20 minutes, take the supernatant into a new centrifuge tube, then add 10 mol/L NaOH adjust 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.

V. ASSAY PROCEDURE

Add following reagents into the 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 °C for 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 protein concentration of sample

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

$$= 0.2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= 0.2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W \times V_{Assay}$$

3. According to the quantity of cells or bacteria

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

$$= 0.2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N \times V_{Assay}$$

4. According to the volume of serum or plasma

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

$$= 0.2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 200 μmol/L = 0.2 μmol/ml;

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

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

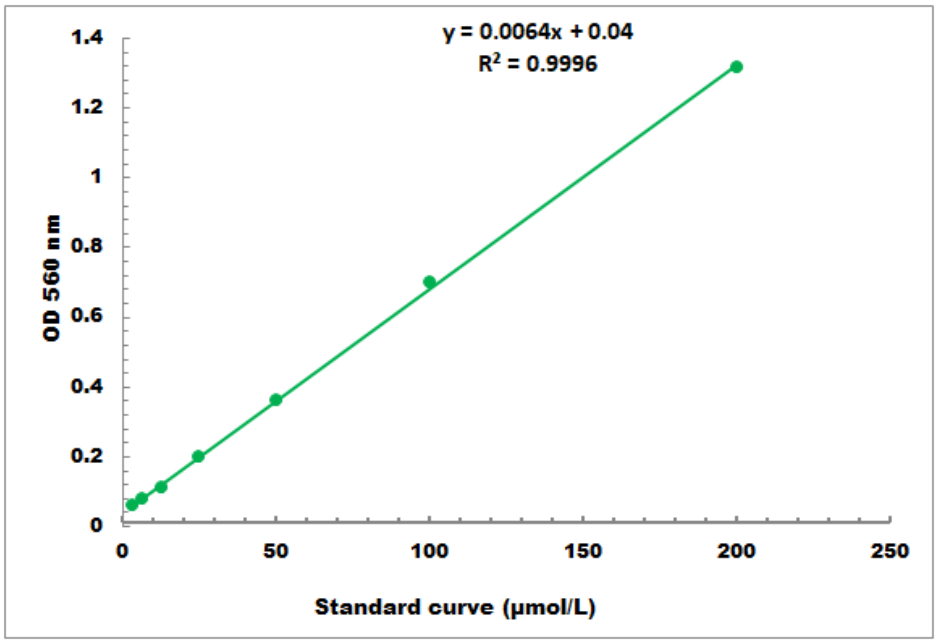
$V_{Standard}$: the volume of standard, 0.04 ml;

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

V_{Assay}: the total volume of HCl and NaOH in sample preparation.

VII. TYPICAL DATA

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



Detection Range: 2 μmol/L - 200 μmol/L

VIII. TECHNICAL SUPPORT

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

IX. NOTES