PRODUCT INFORMATION & MANUAL

Hydroxyproline Assay Kit (Colorimetric)

NBP3-24508

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

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Hydroxyproline Assay Kit (Colorimetric)

Catalog No: NBP3-24508

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.04 μg/mL

Detection range: 0.04-10 μg/mL

Average intra-assay CV (%): 1.2

Average inter-assay CV (%): 4.4

Average recovery rate (%): 101

- This kit is for research use only.
- Instructions should be followed strictly, changes of operation may result in unreliable results.
- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can measure hydroxyproline (HYP) content in serum, animal tissue and urine samples.

▲Background

Hydroxyproline (HYP), one of the sub-amino acids and the main components of collagen tissue, is the unique amino acid in collagen. Collagen tissue is mostly distributed in skin, tendon, blood vessel, cartilage and connective tissue of human body. Many diseases can be accompanied by changes in collagen metabolism, resulting in changes in the content of HYP in blood, urine and tissues. The measurement of HYP can be used to judge the catabolism of connective tissue and the degree of fibrosis in the body.

▲Detection principle

The sample is hydrolyzed to generate free HYP, and hydroxyproline can produce oxidation product under the action of oxidizing agent. The generated oxidation product can react with chromogenic agent to produce burgundy. The concentration of hydroxyproline can be calculated by measuring the OD value at 558 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Oxidant Agent	Powder × 1 vial	2-8℃ , 12 months, shading light
Reagent 2	Buffer Solution	15 mL × 1 vial	2-8℃ , 12 months
Reagent 3	Oxidant Agent Solvent	15 mL × 1 vial	2-8℃ , 12 months
Reagent 4	Chromogenic Agent	Powder × 1 vial	2-8℃ , 12 months, shading light
Reagent 5	Chromogenic Agent Solvent	52 mL × 1 vial	2-8℃ , 12 months
Reagent 6	HYP Standard	5 mg × 2 vials	2-8℃ , 12 months, shading light
Reagent 7	pH Adjusting Solution A	60 mL × 2 vials	2-8℃ , 12 months
Reagent 8	pH Adjusting Solution B	60 mL × 2 vials	2-8℃ , 12 months
Reagent 9	Clarificant	Powder × 2 vials	2-8℃ , 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Vortex mixer, Centrifuge, Water bath, Microplate reader (550-570 nm, optimum wavelength: 558 nm)

Reagents:

6 mol/L Hydrochloric acid, Concentrated hydrochloric acid (12 mol/L), N-propyl alcohol

Consumptive material

Test tube, Glass tube, pH test strips

⚠Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲The key points of the assay

1. Strictly control reaction time and temperature.
2. Adjust the pH value to 6.5-7.0 after sample hydrolysate.
3. Cut the tissue samples. It is recommended to prepare the samples one day in advance when samples are partial, and can be stored at 2-8°C after adjusting pH volume.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 1 working solution:
   Dissolve a vial of reagent 1 with 12 mL of reagent 3 and mix fully, then add 12 mL of reagent 2 and mix fully. The prepared solution can be stored at 2-8°C for 5 days with shading light.
3. Preparation of reagent 4 working solution:
   Dissolve a vial of reagent 4 with 50 mL of reagent 5 and mix fully. The prepared solution can be stored at 2-8°C for 5 days with shading light.
4. Preparation of 1 mg/mL HYP standard:
   Dissolve a vial of reagent 6 with 5 mL of double distilled water and mix fully. The prepared solution can be stored at 2-8°C for 15 days.
5. Preparation of 100 μg/mL HYP standard:
   Dilute 1 mg/mL HYP standard with double distilled water at a ratio of 1:9. Prepare the fresh needed amount before use.

▲ Sample preparation

1. Tissue and urine sample:

Tissue sample hydrolysis: accurately weigh 100 mg tissue sample, cut into pieces and put into a glass tube, add 1 mL of 6 mol/L hydrochloric acid, seal and hydrolyze at 95°C for 6 h.

Urine sample hydrolysis: take 0.5 mL of urine sample into a glass tube, add 0.5 mL of concentrated hydrochloric acid (12 mol/L), seal and hydrolyzed at 95°C for 6 h.

Adjust the pH value of sample hydrolysate: Cool sample hydrolysate with running water, and add 1 mL of reagent 7 and 0.5 mL of reagent 8 and mix fully, and then add reagent 8 drop by drop. Measure the pH value of the solution to 6.5-7.0 using precision pH test paper, add the double distilled wate to a final volume of 10 mL and mix fully.

Decolorization of sample hydrolysate: Take 2 mL sample hydrolysate into the centrifugal tube, add about 20 mg of reagent 9 and mix fully, centrifuge at 1500×g for 10 minutes, then take the supernatant for detection.

2. Serum and plasma samples:

Mix 200 μL of serum sample with 800 μL of n-propanol fully, centrifuge at 4°C at 8000×g for 10 min, and Supplement the supernatant with double distilled water to 1 mL for detection.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.04-10 μg/mL).

The recommended dilution factor for different samples is as follows (for reference only)

Sample type	Dilution factor
10% Rat liver tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Rat lung tissue homogenate	1
10% Rat brain tissue homogenate	1
Chicken Tendon	20-30
Fish scale	20-30
Porcine cartilage	15-25
Human urine	1

Note: The diluent is double distilled water; For little tissue sample, the addition of hydrochloric acid solution, pH adjustment solution and final constant volume can be reduced proportionally. At least 400 μL of sample hydrolysate is required for detection

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

▲Detailed operation steps

1. The preparation of standard curve

Dilute 100 μg/mL standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 1, 2, 3, 4, 6, 8, 10 μg/mL. Reference is as follows:

Number	Standard concentrations (μg/mL)	100 μg/mL standard solution (μL)	Double distilled water (μL)
A	0	0	1000
B	1	10	990
C	2	20	980
D	3	30	970
E	4	40	960
F	6	60	940
G	8	80	920
H	10	100	900

2. The measurement of samples

(1) Standard tube: Take 400 μL of standard solution with different concentrations to the 2 mL EP tube.

Sample tube: Take 400 μL of sample to the 2 mL EP tube.

(2) Add 200 μL of reagent 1 working solution to each tube.

(3) Mix fully and stand at room temperature for 15 min.

(4) Add 400 μL of reagent 4 working solution to each tube.

(5) Mix fully and incubate the tubes at 60℃ for 15 min.

(6) Cool the tubes to room temperature with running water, then take 200 μL to the corresponding wells of microplate.

(7) Measure the OD value of each well at 558 nm with microplate reader.

▲Summary operation table

	Standard tube	Sample tube
Standard solution with different concentrations (μL)	400	
Sample (μL)		400
Reagent 1 working solution (μL)	200	200
Mix fully and stand at room temperature for 15 min..		
Regent 4 working solution (μL)	400	400
Mix fully and incubate the tubes at 60℃ for 15 min. Cool the tubes to room temperature, Then take 200 μL to the corresponding wells of microplate. Measure the OD value of each well.		

Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. Tissue sample:

$$\text{HYP content (μg/mg wet weight)} = (\Delta A - b) \div a \times V \div m \times f$$

2. Urine sample:

$$\text{HYP content (μg/mL)} = (\Delta A - b) \div a \times V \div V_1 \times f$$

3. Serum sample:

$$\text{HYP content (μg/mL)} = (\Delta A - b) \div a \times V_3 \div V_2 \times f$$

Note:

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).

x: The concentration of Standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA:$OD_{Sample} - OD_{Blank.}$

V: The volume of sample hydrolysate after pH adjustmen, 10 mL.

f: Dilution factor of sample before tested.

m: The weight of the sample, mg.

V_1: The volume of urine sample, mL.

V_2: The volume of serum sample, mL.

V_3: The final volume of supernatant of serum sample, mL.

Appendix I Data

▲Example analysis

For fish scale, weigh 98.3 mg fish scale sample, take the hydrolyzed sample and dilute for 25 times, and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.0615 x + 0.0035, the average OD value of the blank is 0.069, the average OD value of the sample is 0.615, and the calculation result is:

HYP content (μg/mg wet weight) = (0.615 – 0.069 – 0.0035) ÷ 0.0615 × 10 × 25 ÷ 98.3 = 22.43 μg/mg wet weight

Appendix II References

1. Lei TW. The Change of Serum Hyp and Pro in Rats after Inject CCl4. Journal of Medical College, 2002.6(27): 221-222.

2. Yu N. Effects of Reduced Glutathione on Contents of Hydroxyproline and Oxidation Stress Reaction in Kidney of Unilateral Ureteral Obstruction in Rat. Chin Crit Care Med, 2007.12(19): 735-738.