（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K062-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（550-570 nm）

Detection range：0.04-10 μg/mL

Elabscience®Hydroxyproline (HYP) Colorimetric Assay Kit (Acid Hydrolysis Method)

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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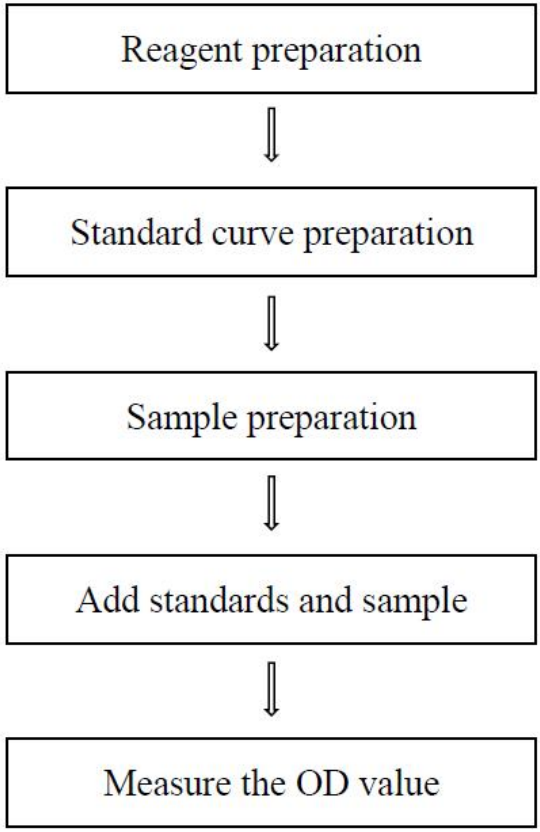
Assay summary



Intended use

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

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	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Oxidant Agent	Powder × 1 vial	Powder × 1 vial	2-8℃, 12 months, shading light
Reagent 2	Buffer Solution	8 mL × 1 vial	15 mL × 1 vial	2-8℃, 12 months
Reagent 3	Oxidant Agent Solvent	8 mL × 1 vial	15 mL × 1 vial	2-8℃, 12 months
Reagent 4	Chromogenic Agent	Powder × 1 vial	Powder × 1 vial	2-8℃, 12 months, shading light
Reagent 5	Chromogenic Agent Solvent	28 mL ×1 vial	52 mL ×1 vial	2-8℃, 12 months
Reagent 6	HYP Standard	5 mg × 1 vial	5 mg × 2 vials	2-8℃, 12 months, shading light
Reagent 7	pH Adjusting Solution A	60 mL × 1 vial	60 mL × 2 vials	2-8℃, 12 months
Reagent 8	pH Adjusting Solution B	60 mL × 1 vial	60 mL × 2 vials	2-8℃, 12 months
Reagent 9	Clarificant	Powder × 1 vial	Powder × 2 vials	2-8℃, 12 months
	Microplate	48 wells	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. For a small volume of reagents, please centrifuge before use, so as not to obtain

sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

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

Reagent preparation

Size 1(48 T):

① Equilibrate all reagents to room temperature before use.

② The preparation of Oxidant working solution:

Dissolve one vial of oxidant agent with 6 mL of oxidant agent solvent, mix well to dissolve. Add 6 mL of buffer solution, mix well to dissolve. Store at 2-8°C for 5 days protected from light.

③ The preparation of Chromogenic working solution:

Dissolve one vial of chromogenic agent with 25 mL of chromogenic agent solvent. Mix well to dissolve. Store at 2-8°C for 5 days protected from light.

④ The preparation of 1 mg/mL HYP standard:

Dissolve one vial of HYP Standard with 5 mL of double distilled water. Mix well to dissolve. Store at 2-8°C for 15 days.

⑤ The preparation of 100 μg/mL HYP standard:

Dilute 40 μL of 1 mg/mL HYP standard with 360 μL of double distilled water, mix well. The 100 μg/mL HYP standard should be prepared on spot.

⑥ The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

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:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μg/mL)	**0**	**1**	**2**	**3**	**4**	**6**	**8**	**10**
100 μg/mL standard solution (μL)	0	10	20	30	40	60	80	100
Double distilled water (μL)	1000	990	980	970	960	940	920	900

Size 2(96 T):

① Equilibrate all reagents to room temperature before use.

② The preparation of oxidant working solution:

Dissolve one vial of oxidant agent with 12 mL of oxidant agent solvent, mix well to dissolve. Add 12 mL of buffer solution, mix well to dissolve. Store at 2-8°C for 5 days protected from light.

③ The preparation of Chromogenic working solution:

Dissolve one vial of chromogenic agent with 50 mL of chromogenic agent solvent. Mix well to dissolve. Store at 2-8°C for 5 days protected from light.

④ The preparation of 1 mg/mL HYP standard:

Dissolve one vial of HYP Standard with 5 mL of double distilled water. Mix well to dissolve. Store at 2-8°C for 15 days.

⑤ The preparation of 100 μg/mL HYP standard:

Dilute 40 μL of 1 mg/mL HYP standard with 360 μL of double distilled water, mix well. The 100 μg/mL HYP standard should be prepared on spot.

⑥ The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

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:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μg/mL)	**0**	**1**	**2**	**3**	**4**	**6**	**8**	**10**
100 μg/mL standard solution (μL)	0	10	20	30	40	60	80	100
Double distilled water (μL)	1000	990	980	970	960	940	920	900

Sample preparation

① Sample preparation

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 0.5 mL of pH Adjusting Solution A and 0.25 mL of pH Adjusting Solution B and mix fully, and then add pH Adjusting Solution B drop by drop. Measure the pH value of the solution to 6.5-7.0 using precision pH test paper. If the pH exceeds 7.0, it can be adjusted to 6.5-7.0 with 2 mol/L hydrochloric acid. Add the double distilled water 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 clarificant and mix fully, centrifuge at 1500×g for 10 minutes, then take the supernatant for detection.

Serum sample:

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.

② Dilution of sample

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). For the dilution of other sample types, please do pretest to confirm the dilution factor

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℃ after adjusting pH volume.

Operating steps

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 oxidant working solution to each tube.

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

④ Add 400 μL of chromogenic 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.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

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]

ΔA: $OD_{Sample} - OD_{Blank}$.

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

f: Dilution factor of sample before test.

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 Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (μg/mL)	1.20	3.50	7.80
%CV	1.5	1.1	1.0

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μg/mL)	1.20	3.50	7.80
%CV	4.2	4.3	4.7

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 101%.

	standard 1	standard 2	standard 3
Expected Conc. (μg/mL)	1.6	3.8	7.4
Observed Conc. (μg/mL)	1.6	3.8	7.7
recovery rate(%)	99	100	104

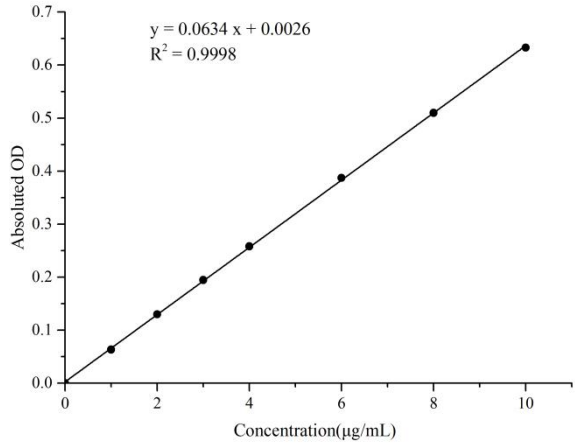
Sensitivity

The analytical sensitivity of the assay is 0.04 μg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (μg/mL)	0	1	2	3	4	6	8	10
Average OD	0.068	0.068	0.068	0.068	0.068	0.068	0.068	0.068
Absoluted OD	0.131	0.131	0.131	0.131	0.131	0.131	0.131	0.131



Appendix II Example Analysis

Example analysis：

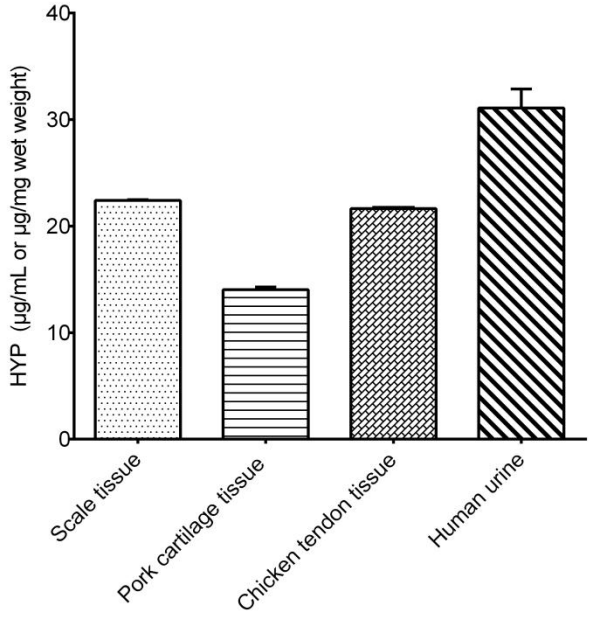
For fish scale, weigh 98.3 mg fish scale sample, take the hydrolyzed sample and dilute for 25 times, and carry the assay according to the operation steps. The results are as follows:

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

Detect fish scale (dilute for 25 times), porcine cartilage (dilute for 20 times), chicken tendon (dilute for 25 times) and human urine according to the protocol, the result is as follows：



Appendix III Publications

1. Yuan L, Liu H, Du X, et al. Airway epithelial ITGB4 deficiency induces airway remodeling in a mouse model[J]. Journal of Allergy and Clinical Immunology, 2023, 151(2): 431-446. e16.
2. Jahan I , Ganesan V , Sahu M ,et al.Adhesivity-tuned bioactive gelatin/gellan hybrid gels drive efficient wound healing[J].International Journal of Biological Macromolecules, 2024, 254(Part2):13.DOI:10.1016/j.ijbiomac.2023.127735.
3. Tawre M S , Padhye A , Chakraborty S ,et al.Bioactive Curcuma aromatica-stabilized silver nanoparticles embedded chitosan dressing with improved antibacterial, anti-inflammatory, and wound healing properties[J].Carbohydrate Polymer Technologies and Applications, 2024, 8.DOI:10.1016/j.carpta.2024.100570.
4. Tirunavalli S K, Andugulapati S B. Geneticin ameliorates pulmonary fibrosis by attenuating the TGF- β /Smad via modulating AMPK/SIRT1 signaling[J]. Life Sciences, 2024, 346: 122626.
5. Zhou S , Wang Z , Gao L ,et al.C5a/C5aR1 axis as a key driver promotes epithelial-to-mesenchymal transition in airway epithelial cells in silica nanoparticles-induced pulmonary fibrosis[J].International Immunopharmacology, 2023, 125.DOI:10.1016/j.intimp. 2023.111112.
6. Kumar R , Kumar A , Mondhe D M ,et al.Antifibrotic Agent Mediated Tumor Microenvironment Modulation and Improved Nanomedicine Delivery in Solid Tumor[J].Molecular Pharmaceutics, 2023, 20(6):2927-2941.DOI:10.1021/ acs.molpharmaceut.2c01081.

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.