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

Catalog No：E-BC-K062-S

Specification：50 Assays(36 samples)/ 100 Assays(86 samples)

Measuring instrument：Spectrophotometer（550-570 nm）

Detection range：0.032-10 μg/mL

Elabscience®Hydroxyproline (HYP) Colorimetric Assay Kit

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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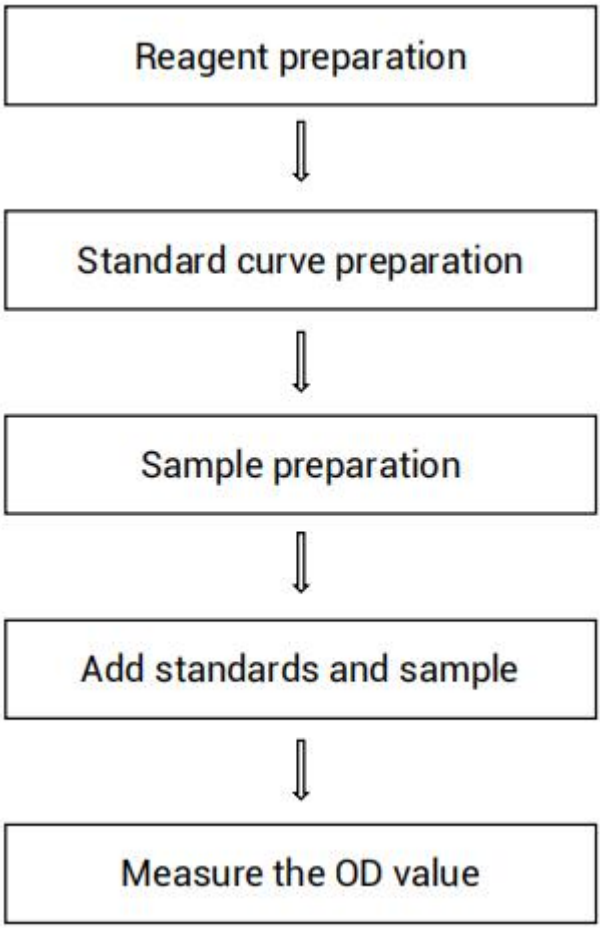
Assay summary



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 (50 Assays)	Size 2 (100 Assays)	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	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

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, Spectrophotometer (550-570 nm, optimum wavelength: 558 nm)

Reagents:

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

Reagent preparation

Size 1(50 Assays)：

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, 3, 4, 6, 8, 10 μg/mL. Reference is as follows:

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

Size 2(100 Assays)：

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 and 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
Concentration (μg/mL)	**0**	**2**	**3**	**4**	**6**	**8**	**10**
100 μg/mL standard solution (μL)	0	20	30	40	60	80	100
Double distilled water (μL)	1000	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 1 mL of pH Adjusting Solution A and 0.5 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, add the double distilled wate to a final volume of 10 mL and mix fully.

Decolorization of sample hydrolysate: Take 1 mL sample hydrolysate into the centrifugal tube, add about 10 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
Chicken cartilage	15-25
Human Urine	1
Fetal bovine serum	1
Rat plasma	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

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 add to quartz cuvette with an optical diameter of 0.5 cm. Set to zero with double distilled water.
⑦ Measure the OD value of each well at 558 nm with spectrophotometer.

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)	0.80	2.60	6.80
%CV	5.3	4.8	4.6

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))	0.80	2.60	6.80
%CV	5.6	6.0	5.5

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 104.9%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μg/mL)	2.5	5.7	8.3
Observed Conc. (μg/mL)	2.7	5.8	8.8
recovery rate(%)	106	102.4	106.3

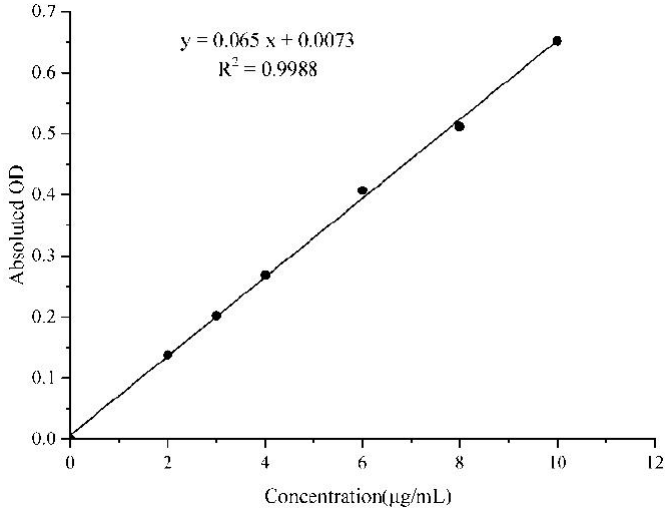
Sensitivity

The analytical sensitivity of the assay is 0.032 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	2	3	4	6	8	10
OD Value	0.005	0.140	0.210	0.278	0.413	0.536	0.669
	0.004	0.146	0.204	0.294	0.410	0.496	0.645
Average OD	0.005	0.143	0.207	0.273	0.412	0.516	0.657
Absoluted OD	0.000	0.139	0.203	0.269	0.407	0.512	0.653



Appendix Π Example Analysis

Example analysis：

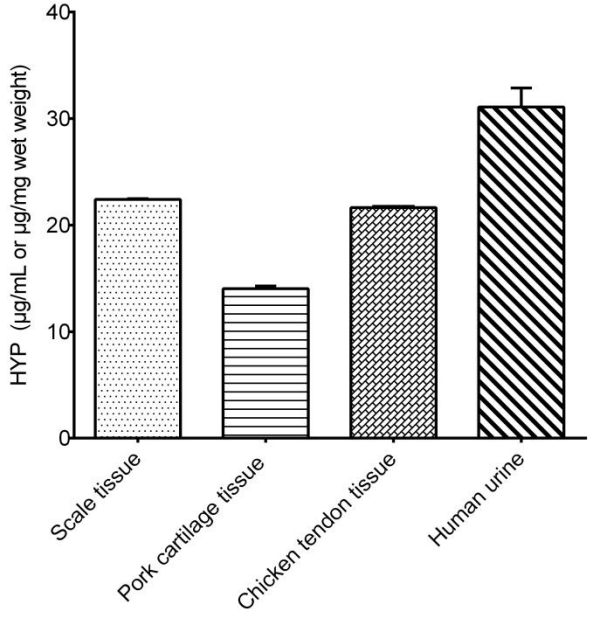
For fish scale, weigh 99.6 mg fish scale sample, take the hydrolyzed sample and dilute for 30 times, and carry the assay according to the operation table. The results are as follows:

standard curve: y = 0.0645 x + 0.0073, the average OD value of the blank is 0.005, the average OD value of the sample is 0.629, and the calculation result is:

HYP content (μg/mg wet weight) = (0.629 – 0.005 – 0.0073) ÷ 0.0645 × 10 × 30 ÷ 99.6

= 29.02 μg/mg wet weight

Detect fish scale (dilute for 30 times), chicken cartilage (dilute for 25 times), chicken tendon (dilute for 30 times) and human urine according to the protocol, the result is as follows：



Appendix III Publications

1. Tirunavalli S K , Kuncha M , Sistla R ,et al.Targeting TGF-β/periostin signaling by sesamol ameliorates pulmonary fibrosis and improves lung function and survival[J].The Journal of Nutritional Biochemistry, 2023:116.DOI:10.1016/j.jnutbio.2023.109294.
2. Ozel C , Apaydin E , Sariboyaci A E ,et al.A multifunctional sateen woven dressings for treatment of skin injuries[J].Colloids and Surfaces, B. Biointerfaces, 2023.DOI:10.1016/j.colsurfb.2023.113197.
3. Cheng L , Zhang S , Zhang Q ,et al.Wound healing potential of silver nanoparticles from Hybanthus enneaspermus on rats[J].Heliyon, 2024, 10(17).DOI:10.1016/j.heliyon.2024.e36118.
4. Banu S A , Pawde A M , Sharun K ,et al.Evaluation of bone marrow-derived mesenchymal stem cells with eggshell membrane for full-thickness wound healing in a rabbit model.[J].Cell and tissue banking, 2023.DOI:10.1007/s10561-023-10105-0.

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.