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

Catalog No：E-BC-K061-M

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

Measuring instrument：Microplate reader(555-565 nm)

Detection range：0.036-5 μg/mL

Elabscience® Hydroxyproline (HYP) Colorimetric Assay Kit (Alkali 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.

Table of contents

Assay summary 3

Intended use 4

Detection principle 4

Kit components & storage 5

Materials prepared by users 6

Reagent preparation 6

Sample preparation 7

Operating steps 8

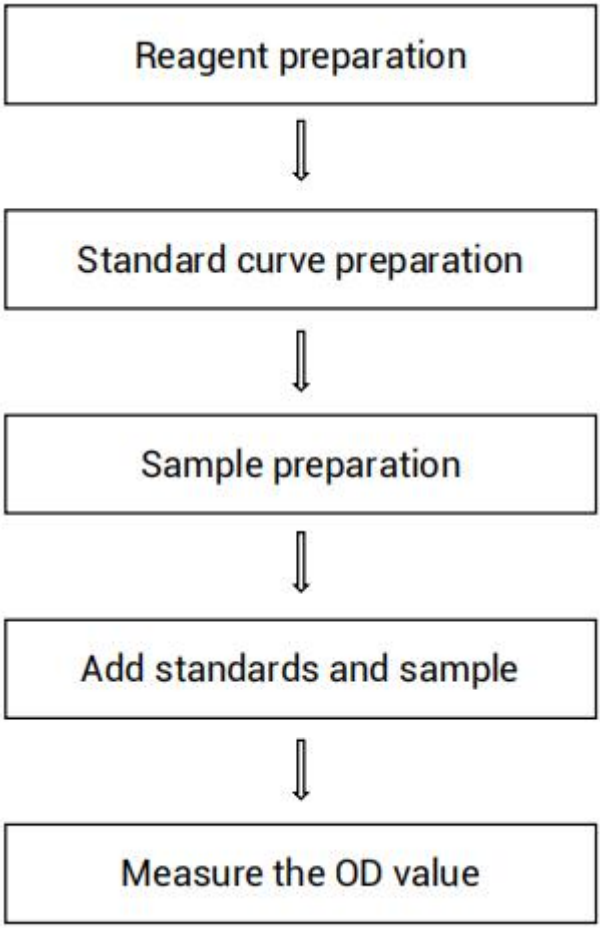
Calculation 9

Appendix I Performance Characteristics 10

Appendix П Example Analysis 12

Statement 13

Assay summary



Intended use

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

Detection principle

Hydroxyproline (HYP) is a derivative of proline, mainly found in collagen. Collagen is the most abundant protein in animals and makes up connective tissues such as skin, bone, cartilage, tendons and ligaments. HYP enhances the crosslinking of collagen fibers through the hydroxyl group in its structure, thus improving the stability and tensile strength of collagen. Since HYP almost only exists in collagen, its content can reflect the synthesis and catabolism of collagen. Therefore, the detection of HYP level can be used as an indicator to monitor collagen metabolism.

The detection principle of this kit: the sample is hydrolyzed by the alkali reagent to generate free HYP, and the oxidation product produced under the action of the oxidant reacts with the color agent to be purple red. The substance has an absorption peak at 565 nm, and within a certain range, its absorbance is linear with the concentration. By measuring the absorbance at 565 nm, the HYP content in the sample can be calculated.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	15 mL × 1 vial	30 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 2	Alkali Reagent	50 mL × 1 vial	50 mL × 2 vials	2-8℃, 12 months, shading light
Reagent 3	Oxidant Solution	15 mL × 1 vial	30 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 4	Chromogenic Agent	30 mL × 1 vial	60 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 5	Stop Reagent	30 mL ×1 vial	60 mL ×1 vial	2-8℃, 12 months, shading light
Reagent 6	Clarificant	6 g × 1 vial	6 g × 2 vials	2-8℃, 12 months, shading light
Reagent 7	Acid Reagent	50 mL × 1 vial	50 mL × 2 vials	2-8℃, 12 months, shading light
Reagent 8	100 μg/mL Standard Solution	1.5 mL × 1 vial	1.5 mL × 2 vials	2-8℃, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

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:

Microplate reader (555-565 nm, optimum wavelength: 565 nm), Water bath

Consumptive material:

Precision pH test paper

Reagent preparation

① Equilibrate all reagents to 25°C before use.

② The preparation of measuring working solution：

For each well, prepare 500 μL of measuring working solution (mix well 250 μL of buffer solution and 250 μL of oxidant solution). The measuring working solution should be prepared on spot and used up within the same day.

③ The preparation of 5 μg/mL standard solution：

Before testing, please prepare sufficient 5 μg/mL standard solution. For example, prepare 13 mL of 5 μg/mL standard solution (mix well 0.65 mL of 100 μg/mL standard solution and 12.35 mL of double distilled water). The 5 μg/mL standard solution should be prepared on spot and used up within the same day.

④ The preparation of standard curve：

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

Dilute 5 μg/mL standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 1, 1.5, 2, 3, 3.5, 4, 5 μg/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μg/mL)	**0**	**1**	**1.5**	**2**	**3**	**3.5**	**4**	**5**
5 μg/mL standard solution (mL)	0	0.6	0.9	1.2	1.8	2.1	2.4	3.0
Double distilled water (mL)	3.0	2.4	2.1	1.8	1.2	0.9	0.6	0

Sample preparation

① Sample preparation

a. Sample hydrolysis:

Tissue sample hydrolysis: accurately weigh 100 mg tissue sample, cut into pieces and put into a glass tube, add 1 mL of alkali reagent, Cover the tube mouth with plastic wrap and open small holes for ventilation, hydrolyze at 95°C for 20 min.

Serum and plasma samples hydrolysis: Add 1 mL of sample and 1 mL of alkali reagent into tube. Cover the tube mouth with plastic wrap and open small holes for ventilation, hydrolyze at 95°C for 20 min.

b. Adjust the pH of the sample hydrolysate:

Cool sample hydrolysate with running water and add 1 mL of acid reagent, mix fully. Add alkali reagent or acid reagent drop by drop. Measure the pH value of the solution to 6.5 using precision pH test paper. Add the double distilled water to a final volume of 10 mL and mix fully.

c. Decolorization of sample hydrolysate:

Take 1.5 mL sample hydrolysate into the centrifugal tube, add about 15 mg of clarificant and mix fully, centrifuge at 15000×g for 10 min, then take the supernatant for detection.

② Dilution of sample

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

Sample type	Dilution factor
Human serum	1
Rat serum	1
Rat plasma	1
Fish scale	4-10
Fin tissue	4-10
Fishtail tissue	4-10
Mouse lung tissue	1
Mouse muscle tissue	2-5
Mouse leg bone tissue	4-10

Note: The diluent is double distilled water. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard tube: Take 1 mL of standard solution with different concentrations to the 5 mL EP tube.
Sample tube: Take 1 mL of sample to the 5 mL EP tube.

② Add 0.5 mL of measuring working solution to each tube. Mix fully and stand at room temperature for 20 min.

③ Add 0.5 mL of stop reagent to each tube. Mix fully and stand at room temperature for 5 min.

④ Add 0.5 mL of chromogenic agent to each tube. Mix fully and water bath at 65°C for 15 min.

⑤ Cool the tubes to room temperature with running water, then take 0.2 mL to the corresponding wells of microplate. Measure the OD value of each well at 565 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. Serum and plasma samples:

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

2. Tissue sample:

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

[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 serum (plasma) 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)	1.00	2.00	4.00
%CV	1.4	1.6	2.1

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)	1.00	2.00	4.00
%CV	2.4	3.1	3.4

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%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (μg/mL)	1.00	2.00	4.00
Observed Conc. (μg/mL)	0.95	1.92	3.92
recovery rate(%)	95	96	98

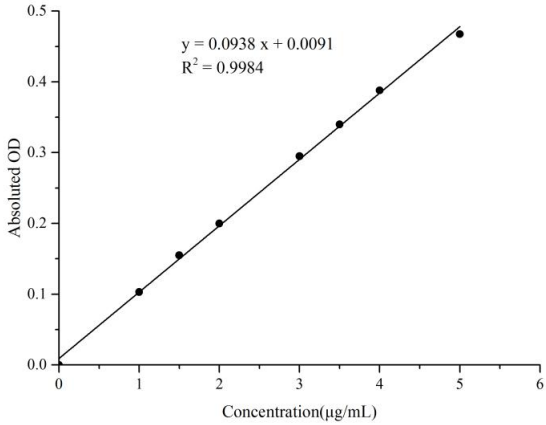
Sensitivity

The analytical sensitivity of the assay is 0.036 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	1.5	2	3	3.5	4	5
OD value	0.081	0.185	0.236	0.282	0.374	0.424	0.472	0.555
	0.081	0.183	0.236	0.280	0.378	0.418	0.466	0.542
Average OD value	0.081	0.184	0.236	0.281	0.376	0.421	0.469	0.549
Absoluted OD value	0	0.103	0.155	0.200	0.295	0.340	0.388	0.468



Appendix Π Example Analysis

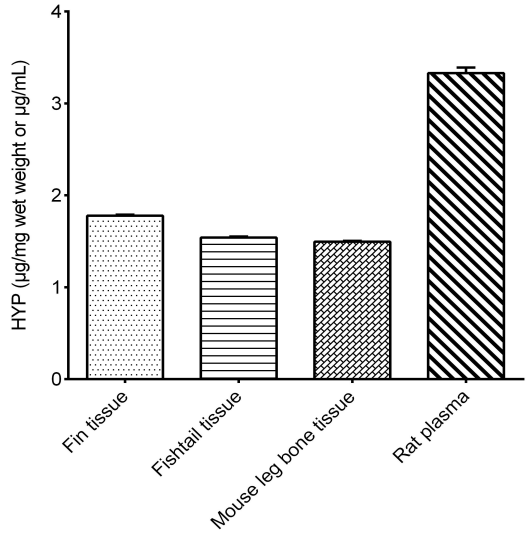
Example analysis：

Take 1 mL of fin tissue which dilute for 4 times and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.0938 x + 0.0091, the average OD value of the blank well is 0.081, the average OD value of the sample well is 0.507, ΔA = 0.507 - 0.081 = 0.426, and the calculation result is:

$$\text{HYP content (μg/mg wet weight)} = \frac{0.426 - 0.0091}{0.0938} \times 10 \times 4 \div 100 = 1.78 \text{ μg/mg wet weight}$$

Detect fin tissue (The wet weight of sample is 100 mg and the constant volume is 10 mL, dilute for 4 times), fishtail tissue (The wet weight of sample is 100 mg and the constant volume is 10 mL, dilute for 4 times), mouse leg bone tissue (The wet weight of sample is 100 mg and the constant volume is 10 mL, dilute for 4 times) and rat plasma (The volume of sample is 1 mL and the constant volume is 10 mL) according to the protocol, the result is as follows：



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.