

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

Catalog No: E-BC-K061-S

Specification: 50 Assays(48 samples)

Measuring instrument: Spectrophotometer

Detection range: 0.01-20 µg/mL

Elabsience[®]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

Tel: 1-832-243-6086

Fax: 1-832-243-6017

Email: techsupport@elabsience.com

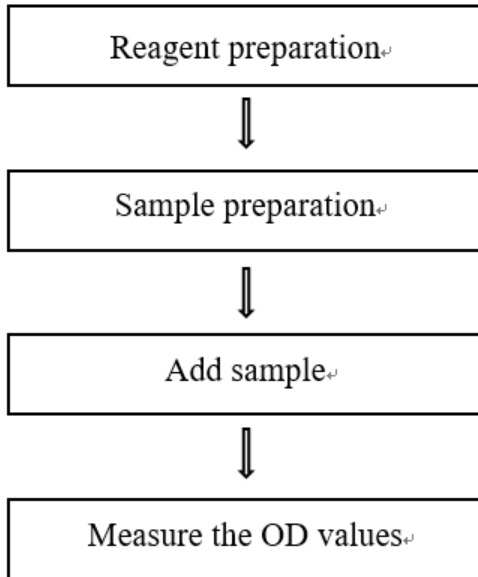
Website: www.elabsience.com

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 be used for detection of hydroxyproline content in samples, such as animal serum (plasma), tissue, culture supernatant and body fluids etc.

Detection principle

The oxidation product which produced by hydroxyproline under the action of oxidant react with dimethylaminobenzaldehyde and show a purplish red color. The content of hydroxyproline can be calculated by measuring the OD value at 550 nm.

Kit components & storage

Item	Component	Size (50 Assays)	Storage
Reagent 1 (A)	Powder	1 vial	2-8 °C, 12 months
Reagent 1 (B)	Solution A	10 mL × 1 vial	2-8 °C, 12 months
Reagent 1 (C)	Solution B	20 mL × 1 vial	2-8 °C, 12 months
Reagent 2	Liquid	30 mL × 1 vial	2-8 °C, 12 months, shading light
Reagent 3 (A)	Powder	1 vial	2-8 °C, 12 months
Reagent 3 (B)	Liquid	30 mL × 1 vial	2-8 °C, 12 months, shading light
Reagent 4	Hydroxyproline Standard	5 mg × 3 vials	2-8 °C, 12 months
Reagent 5	Hydrolyzed Solution	60 mL × 1 vial	2-8 °C, 12 months
Reagent 6	Indicator	5 mL × 1 vial	2-8 °C, 12 months
Reagent 7	PH Adjusted Liquid A	60 mL × 1 vial	2-8 °C, 12 months
Reagent 8	PH Adjusted Liquid B	30 mL × 1 vial	2-8 °C, 12 months
Reagent 9	Acticarbon	1 vial	2-8 °C, 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:

Spectrophotometer (550 nm), Micropipettor, Water bath, Incubator, Vortex mixer, Centrifuge

Reagent preparation

- ① Equilibrate all reagents to room temperature before use.
- ② The preparation of reagent 1 :
Dissolve a vial of powder with 10 mL of Solution A fully and then mix with 20 mL of Solution B. Mix fully and store at 2-8 °C for 3 months.
Note: The powder must be completely dissolved before adding solution B.
- ③ The preparation of reagent 3 application solution :
Dissolve a vial of powder with 30 mL of Reagent 3 (B) fully. Prepared before use. It can be store at 2-8 °C for a month with shading light.
- ④ The preparation of 100 µg/mL hydroxyproline standard application solution :
Dissolve a vial of powder with double distilled water fully and add double distilled water to a final volume of 50 mL. It can be store at 2-8 °C for 2 weeks.
- ⑤ The preparation of 5 µg/mL hydroxyproline standard application solution :
Take 1 mL of 100 µg/mL hydroxyproline standard application solution and add double distilled water to a final volume of 20 mL. Prepare fresh solution before use.

Sample preparation

① Hydrolysis of sample

Serum (plasma): Take 0.5 mL serum (plasma) into a tube and add exactly 1 mL hydrolyzed solution, mix fully. Cover the lid and incubate at 95 °C water bath or boiling water for 20 min.

Urine (culture supernatant): Take 1 mL urine (or 0.5 mL culture supernatant) into a tube and add exactly 1 mL hydrolyzed solution, mix fully. Cover the lid and incubate at 95 °C water bath or boiling water for 20 min.

Tissue: Weigh accurately 30~100 mg wet tissue into a tube and add exactly 1 mL hydrolyzed solution, mix fully. Cover the lid and incubate at 95 °C water bath or boiling water for 20 min (shake the tube to mix at 10 min to hydrolyze fully).

Recommended wet weight of tissue sample: skin tissue--0.03-0.05 g, cartilaginous tissue/ liver tissue --0.08-0.1 g.

② Adjust pH to 6.0~6.8:

- 1) Cool the hydrolyzed sample under running water, then add 10 μ L of indicator and mix fully.
- 2) Add 1 mL of pH adjusted liquid A accurately and mix fully (At this time the solution is red).
- 3) Carefully add pH adjusted liquid B a drop by a drop, mix fully after adding each drop, until the red is disappeared (At this time the pH is 6.0-6.8).
- 4) Add double distilled water to a final volume of 10 mL and mix fully.
- 5) Take 3-4 mL diluent hydrolyzed liquid, add 20-30 mg acticarbon (the supernatant is clarified colorless after centrifugation), mix fully, centrifuge at 3500 rpm for 10 min, take 1 mL supernatant for test.

Operation table

	Blank tube	Standard tube	Sample tube
Double distilled water (mL)	1.0		
5 µg/mL hydroxyproline standard application solution (mL)		1.0	
Prepared sample (mL)			1.0
Reagent 1 (mL)	0.5	0.5	0.5
Mix fully and stand for 10 min at room temperature			
Reagent 2 (mL)	0.5	0.5	0.5
Mix fully and stand for 5 min at room temperature			
Reagent 3 application solution (mL)	0.5	0.5	0.5
Mix fully and incubate in 60 °C water bath for 15 min. Cool with running water, then centrifuge at 3500 rpm for 10 min and take the supernatant (Be careful not to mix with precipitation below). Set spectrophotometer to zero with double distilled water and measure the OD values of each tube at 550 nm with 1 cm optical path cuvette.			

Calculation

The sample:

1. Serum (plasma), urine, culture supernatant sample:

$$\text{Hydroxyproline content } (\mu\text{g/mL}) = \frac{\Delta A_1}{\Delta A_2} \times c \times f \times \frac{V_{\text{Total}}}{V_{\text{Sample}}}$$

2. Tissue sample:

$$\text{Hydroxyproline content } (\mu\text{g/mg wet tissue}) = \frac{\Delta A_1}{\Delta A_2} \times c \times f \times \frac{V_{\text{Total}}}{m}$$

[Note]

ΔA_1 : OD_{Sample} - OD_{Blank}.

ΔA_2 : OD_{Standard} - OD_{Blank}.

c: Concentration of standard (5 $\mu\text{g/mL}$).

f: Dilution factor of sample before test.

V_{Total} : Total volume of hydrolysis solution (10 mL).

V_{Sample} : Total volume of sample (mL).

m: The wet weight of tissue (mg).

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.

