Technical Manual

Hydroxyproline (Hyp) Colorimetric Assay Kit (Alkali hydrolysis Method)

- Catalogue Code: MAES0067
- Size: 50 Assays
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

50 Assays

Range:

0.01-20µg/mL

Sensitivity:

0.01µg/mL

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Hydroxyproline accounts for 13.4% in collagen, minute quantity in elastin, and not exists in other proteins. And collagen mostly exists in skin, tendon, cartilage, blood vessel, etc, so the quantity and energy of hydroxyproline can reflect collagen metabolic status of connective tissue diseases. Surgical trauma can lead to the increase of hydroxyproline excretion amount in urine. Fiber can increase through inflammation because of high fat, low protein, alcoholism and poison, malnutrition or hepatocellular degeneration and necrosis, then segments hepatic lobule and results in liver cirrhosis. When affected with hepatic fibrosis, collagenous fiber is the main increasing component in liver. And hydroxyproline is specific to collagenous fiber. So the content of liver collagen can be calculated by measuring the content of liver hydroxyproline to reflect the degree of hepatic fibrosis. The decomposition of collagen fiber primarily depends on the function of collagenase and other proteases, whose decomposition product - hydroxyproline is excreted in the urine. So measuring the content of urine hydroxyproline can reflect the degradation situation of collagen. On the one hand, measuring the content of hydroxyproline in tissue and urine can judge the degree of fibrosis; on the other hand, it can screen the drugs of prevention and treatment.

3. Intended Use

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

4. 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.

5. Kit Components & Storage

Item	Specification	Storage
Powder A	1 vial	2-8°C, 6 months
Solution A	10 mL × 1 vial	2-8°C, 6 months
Solution B	20 mL × 1 vial	2-8°C, 6 months
Liquid 1	30 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Powder B	1 vial	2-8°C, 6 months
Liquid 2	30 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Hydroxyproline Standard	5 mg × 3 vials	2-8°C, 6 months
Hydrolysed Solution	60 mL × 1 vial	2-8°C, 6 months
Indicator	5 mL × 1 vial	2-8°C, 6 months
pH Adjusted Liquid A	60 mL × 1 vial	2-8°C, 6 months
pH Adjusted Liquid B	30 mL × 1 vial	2-8°C, 6 months
Acticarbon	1 vial	2-8°C, 6 months

Materials required but not supplied

- Micropipette
- Vortex mixer
- Water bath
- Spectrophotometer (550 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

6. Reagent Preparation:

1. Preparation of **working solution A:** Dissolve a vial of powder A with a vial of solution A and then mix with a vial of solution B. Mix fully and store at 2-8°C for 3 months. **Note:** Powder A must be completely dissolved before adding solution B.

2. Preparation of **working solution B:** Dissolve a vial of powder B with 30 mL of liquid 2 and mix fully. The prepared solution can be stored at 2-8°C for a month away from direct sunlight.

3. Preparation of **100 µg/mL hydroxyproline standard solution:** Dissolve a vial of standard powder with double distilled water and add double distilled water to a final volume of 50 mL. It can be stored at 2-8°C for 2 weeks.

4. Preparation of **5 µg/mL hydroxyproline standard solution:** Take 1 mL of 100 µg/mL hydroxyproline standard and add double distilled water to a final volume of 20 mL. Prepare fresh solution before use.

7. 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 in a water bath or in 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 in a water bath or in boiling water for 20 min.

Tissue: Weigh 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 in a water bath or in 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 µL of indicator and mix fully.

2) Add 1 mL of pH adjusted liquid A and mix fully (At this time the solution is red).

3) Carefully add pH adjusted liquid B drop by a drop, mix fully after adding each drop, until the red has 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.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.01-20µg/mL).

8. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 550 nm

9. Operation Steps

Operation Table

	Blank tube	Standard tube	Sample tube
Double distilled water (mL)	1.0		
Hydroxyproline standard solution (mL) 5 μg/mL		1.0	
Prepared sample (mL)			1.0
Working solution, A (mL)	0.5	0.5	0.5
Mix fully and stand for 10 min at room temperature			
Liquid 1 (mL)	0.5	0.5	0.5
Mix fully and stand for 5 min at room temperature			
Working solution B (mL)	0.5	0.5	0.5
Mix fully and incubate in a 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.			

10. Calculations

1. **Serum (plasma) and other liquid sample:**

$$\text{Hydroxyproline content (μg/mL)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f \times \frac{V_{total}}{V_{sample}}$$

2. **Tissue and cell samples:**

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

ΔA_1: $OD_{Sample} - OD_{Blank}$

ΔA_2: $OD_{Standard} - OD_{Blank}$

c: Concentration of standard, 5 μg/mL

f: Dilution factor of sample before test

V_{total}: The volume of sample hydrolysate after pH adjustmen, 10 mL

V_{sample}: The volume of sample, mL

m: The weight of the sample, mg

11. Performance Characteristics

Detection Range	0.01-20μg/mL
Sensitivity	0.01μg/mL

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

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