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Hydroxyproline Assay Kit

Cat#: orb1499930 (manual)

Size: 100T/96S

Micro-assay

Product composition and storage conditions :

No.	Specifications	Storage Conditions
Extraction solution	6mol/L Hydrochloric acid (self- provided)	Concentrated hydrochloric acid (37%): H2O (V/V) = 1:1, stored at room temperature.
orb1499930-A	A1: Powder ×1	4°C; Add 10mL A2 to A1 for full dissolution (make sure it is completely dissolved); Then add 20mL A3 and mix well. The prepared reagent can be stored for 3 months at 4°C.
orb1499930-A	A2: 10ml ×1	
orb1499930-A	A3: 20ml ×1	
orb1499930- B	30ml ×1	4°C; Protected from light.
orb1499930- C	C1: Powder ×1	4°C; Add 30mL C2 in C1 for full dissolution. The prepared reagent can be stored in the dark for a month at 4°C.
orb1499930- C	C2: 30ml ×1	
orb1499930- Standard (5mg/tube)	Powder ×1	4 °C; Add 1ml double distilled water in standard to prepare 5 mg/mL standard stock solution before use, which can be stored for 2 weeks at 4 °C.

※Before the formal measurement, be sure to take 2-3 samples with large expected differences for predetermination.

Introduction:

Significance: Hydroxyproline (HYP) is one of the main components of collagen in the body, and collagen is mostly distributed in the skin, tendons, cartilage and blood vessels, so HYP content is an important indicator to reflect the degree of collagen tissue metabolism and fibrosis.

Principle: The oxidation product produced by hydroxyproline under the action of oxidant and dimethylaminobenzaldehyde is purple-red, and its content can be deduced according to the shade of its color.

Own supplies:

Visible spectrophotometer/microplate reader, micro quartz cuvette/96-wells plate, water bath, balance, ovens, glass tubes, centrifuge, adjustable pipette , mortar or homogenizer, 6mol/L hydrochloric acid, 10mol/L NaOH and distilled water.

Hydroxyproline extraction:

1. **Tissue:** Weigh about 0.2 g of sample into a glass tube, and cut the tissue as much as possible for digestion. The lid is slightly loose and not sealed. Then add 2 mL of extracting solution, boil or digest in an oven at 110 ° C for 2h to 6h until there is no visible large lump. After cooling, adjust the pH value to 6-8 with 10mol/L NaOH (about 1 mL), and then dilute to 4mL with distilled water. Finally, centrifuge for 20 min at 16000 rpm, 25°C (if impurities are still present after centrifugation, they can be removed by filtration). Take the supernatant for test (black substance may be formed in the process, if it cannot be digested for a long time, it may be carbonized substance, which does not affect the experiment).

2. **Cells:** Take 5 million cells, add 1 mL of extracting solution, boil or digest in an oven at 110 ° C for 2 to 6 hours to be transparent, adjust the pH value to 6-8 with 10mol/L NaOH (about 0.5 mL) after cooling, dilute to 2 mL with the distilled water, centrifuge for 20 min at 16000 rpm, 25°C, and take supernatant for test.

Measurement steps:

1. Preheat the UV spectrophotometer or microplate reader for at least 30 minutes, adjust the wavelength to 560nm, set zero with the distilled water.

2. Dilute the standard with distilled water to a standard solution of 10,7.5, 5,2.5, 1.25, 0.625μg/mL.

3. Add the following reagents in sequence to the micro quartz cuvette or microplate :

Reagent name	Blank tube (μl)	Measuring tube (μl)	Standard tube (μl)
Distilled water	100		
Sample		100	
Standard			100
orb1499930-A	50	50	50
Mix well and allow to stand at room temperature for 10 min			
orb1499930-B	50	50	50
Mix well and allow to stand at room temperature for 5 min			
orb1499930-C	50	50	50
Mix evenly, place in 60° C water bath for 20 min, take out and allow to stand at RT for 15 min, centrifuge for 10 min at 3500 rpm, take 200 μL in/96-well plate of microglass cuvette, detect absorbance at 550nm, calculate ΔA measuring =A measuring -A blank, ΔA standard =A standard-A blank.			

Calculation of hydroxyproline content:

1. **Drawing of standard curve:** Draw the standard curve with the concentration of standard as the x-axis and the ΔA standard (ΔA standard =A standard-A blank) as the y-axis to obtain Equation $y=kx+b$. The ΔA measuring (ΔA measuring =A measuring -A blank) was carried into the equation to

obtain x (µg/mL).

2. **Calculation of hydroxyproline content:**

(1) Calculated by sample weight

Hydroxyproline content in tissue (ug/g) = $x \times V$ sample $\div (W \times V$ sample $\div V$ extraction) $= 4x \div W$

(2) Calculated by sample protein concentration

Hydroxyproline content in tissue (µg/mg prot) = $x \times V$ sample $\div (Cpr \times V$ sample) $= x \div Cpr$

(3) Calculated by bacterial or cell density

Hydroxyproline content in cells (µg/10^4 cell = $x \times V$ sample $\div$ (number of cells $\times V$ sample $\div V$ cell extract) $= 2x \div$ number of cells

Note: V sample: Sample volume added, 0.05mL; V extraction: Volume of tissue extract, 4mL; V cell extraction: Volume of cell extract, 2mL; W: Sample weight, g; Number of cells: 10^4; Cpr: Protein concentration of sample, mg/mL.

Precautions:

1. Reagent has certain toxicity. Please take protective measures during operation to prevent inhalation or contact with skin.

2. If the measured absorbance exceeds the linear range, increase the sample size or dilute the sample before measurement.

3. When calculated by sample protein concentration, the protein in the sample is extracted separately and determined.

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