Hydroxyproline Assay Kit

Catalog No.: abx298833

Size: 96 tests

Detection Range: 0.04 µg/ml – 10 µg/ml

Sensitivity: 0.04 µg/ml

Storage: Store all components at 4°C. Store the Oxidant, Chromogenic Reagent, and Standard in the dark.

Application: For detection and quantification of Hydroxyproline content in serum, urine, and tissue homogenates.

Introduction

Abbexa's Hydroxyproline Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Hydroxyproline content. Samples are hydrolyzed to produce free Hydroxyproline, which is then oxidized. The oxidation product reacts with the Chromogenic Reagent to produce a red-colored product which has an absorbance maximum at 558 nm. The intensity of the color is proportional to the Hydroxyproline concentration, which can then be calculated.

Kit components

1. 96-well microplate
2. Oxidant: 1 × vial
3. Buffer Solution: 1 x 15 ml
4. Oxidant Reagent Solvent: 1 x 15 ml
5. Chromogenic Reagent: 1 × vial
6. Chromogenic Reagent Solvent: 52 ml
7. Standard: 2 × vials
8. pH Adjusting Solution A: 2 × 60 ml
9. pH Adjusting Solution B: 2 × 60 ml
10. Clarificant: 2 × vials
11. Plate Sealer: 2

Materials required but not provided

1. Microplate reader (558 nm)
2. Distilled water
3. 6 mol/L Hydrochloric acid
4. Concentrated Hydrochloric acid (12 mol/L)
5. N-propanol
6. Pipette and pipette tips
7. Glass tubes
8. 2 ml microcentrifuge tubes
9. Centrifuge
10. Vortex mixer
11. Water bath
12. pH test strips

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used:

- **Serum:** Add 800 µl n-propanol to 200 µl serum and mix fully, then centrifuge at 8000 × g at 4 °C for 10 minutes. Collect the supernatant and increase the volume to 1 ml with double distilled water.
- **Urine:** In a glass tube, add 500 µl concentrated hydrochloric acid (12 mol/L) to 500 µl urine. Seal the tube then hydrolyze at 95 °C for 6 hours.
    - **pH Adjustment:** Cool sample hydrolysate with running water then add 1 ml pH Adjusting Solution A and 0.5 ml pH Adjusting Solution B and mix fully. Measure the pH, then add pH Adjusting Solution B one drop at a time until pH is adjusted to 6.5 – 7.0. Increase the volume to 10 ml with double distilled water and mix fully.
    - **Decolorization:** Take 2 ml of sample hydrolysate, add 20 mg of Clarificant and mix fully. Centrifuge at 1500 × g for 10 minutes, then collect the supernatant for assay.
- **Tissue Homogenates:** Carefully weigh 0.1 g of tissue, mince and transfer to a glass tube. Add 1 ml of 6 mol/L hydrochloric acid. Seal the tube then hydrolyze at 95 °C for 6 hours.
    - **pH Adjustment:** Cool sample hydrolysate with running water then add 1 ml pH Adjusting Solution A and 0.5 ml pH Adjusting Solution B and mix fully. Measure the pH, then add pH Adjusting Solution B one drop at a time until pH is adjusted to 6.5 – 7.0. Increase the volume to 10 ml with double distilled water and mix fully.
    - **Decolorization:** Take 2 ml of sample hydrolysate, add 20 mg of Clarificant and mix fully. Centrifuge at 1500 × g for 10 minutes, then collect the supernatant for assay.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with double distilled water, then carry out the assay procedure. 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
Pig cartilage	15 – 25
Human urine	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.

2. Reagents

- **Oxidant Working Solution**: Dissolve vial of Oxidant with 12 ml of Oxidant Reagent Solvent and mix fully, then add 12 ml of Buffer Solution and mix fully. Prepare fresh, solution may be stored at 4 °C for up to 5 days in the dark.
- **Chromogenic Reagent Working Solution**: Dissolve a vial of Chromogenic Reagent with 50 ml of Chromogenic Reagent Solvent and mix fully. Prepare fresh, solution may be stored at 4 °C for up to 5 days in the dark.
- **Standard Solution (1 mg/ml)**: Dissolve a vial of Standard with 5 ml of double distilled water and mix fully. Prepare fresh, solution may be stored at 4 °C for up to 15 days.
- **Standard Solution (100 µg/ml):** Dilute 1 mg/ml Standard Solution with double distilled water to a ratio of 1:9. Prepare fresh and use immediately.

Note:

- Allow all reagents to equilibrate to room temperature before use.

B. Assay Procedure

1. Dilute 100 µg/ml standard solution with double distilled water to produce a standard dilution gradient as detailed in the following table:

	Standard Concentration (µg/ml)	100 µg/ml standard solution (µl)	Double distilled water (µl)
A	0	0	1000
B	1	10	990
C	2	20	980
D	3	30	970
E	4	40	960
F	6	60	940
G	8	80	920
H	10	100	900

2. Add 400 µl diluted standard to 2 ml standard tubes.
3. Add 400 µl sample to 2 ml sample tubes
4. Add 200 µl Oxidant to each tube and mix fully, then incubate at room temperature for 15 minutes.
5. Add 400 µl Chromogenic Reagent to each tube and mix fully, then incubate at 60 °C for 15 minutes.
6. Cool the tubes to room temperature with running water, then transfer 200 µl to a microplate for assay.
7. Measure absorbances at 558 nm.

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula:

$$y = ax + b$$

Note:

- The blank is the 0 µg/ml standard.
- It is important to adhere strictly to the stated reaction times and temperatures.

Based on this curve, the concentration of Hydroxyproline in each sample well can be derived with the following formulae:

1. **Serum samples:**

$$\textbf{Hydroxyproline content } (\mu g/ml) = \frac{\Delta A - b}{a} \times \frac{V_3 \times f}{V_2}$$

2. **Urine samples:**

$$\textbf{Hydroxyproline content } (\mu g/ml) = \frac{\Delta A - b}{a} \times \frac{V \times f}{V_1}$$

3. **Tissue Homogenates**

$$\textbf{Hydroxyproline content } (\mu g/ml \textbf{ wet weight}) = \frac{\Delta A - b}{a} \times \frac{V \times f}{m}$$

where:

y	$OD_{Standard} - OD_{Blank}$
x	The concentration of standard
a	The slope of the standard curve
b	The intercept of the standard curve
ΔA	$OD_{Sample} - OD_{Blank}$
V	Sample hydrolysate volume after pH adjustment (10 ml)
f	Sample dilution factor
m	Sample weight (mg)
V_1	Urine sample volume (ml)
V_2	Serum sample volume (ml)
V_3	Final volume of serum sample supernatant (ml)