Hydroxyproline Assay Kit

Catalog No.: abx294049

Size: 50 tests

Detection Range: 0.024 5 µg/ml – 5 µg/ml

Sensitivity: 0.024 µl/ml

Storage: Store all components at 4°C in the dark.

Application: For detection and quantification of Hydroxyproline content in serum, plasma, and tissue samples.

Introduction

Abbexa's Hydroxyproline Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Hydroxyproline content. Samples are hydrolyzed using an Alkaline Reagent to produce free Hydroxyproline, which is then oxidized using an Oxidant Solution. The oxidation product reacts with the Chromogenic Reagent to produce a purple-red colored product which has an absorbance maximum at 565 nm. The intensity of the color is proportional to the Hydroxyproline concentration, which can then be calculated.

Kit components

1. Buffer Solution: 15 ml
2. Alkaline Reagent: 50 ml
3. Acidic Reagent: 50 ml
4. Oxidant Solution: 15 ml
5. Chromogenic Reagent: 30 ml
6. Stop Reagent: 30 ml
7. Clarifying Reagent: 6 g
8. 100 µg/ml Standard Solution: 1.5 ml

Materials Required But Not Provided

1. Spectrophotometer (565 nm)
2. Double-distilled water
3. pH test paper
4. Pipette and pipette tips
5. Sterile scalpels
6. Glass tubes
7. Plastic tubes
8. Centrifuge tubes
9. Parafilm
10. Water bath
11. Centrifuge
12. Vortex mixer
13. Quartz cuvette (1cm optical path).

Protocol

1. Preparation of samples and reagents

Isolate the test samples soon after collecting and analyze immediately or 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.

1. **Sample Hydrolysis:**
   - **Tissue:** Carefully weigh 100 mg of tissue and cut into small pieces using a sterile scalpel. Place the tissue into a glass tube and add 1 ml of Alkaline Reagent. Cover the tube with a plastic wrap and create small holes for ventilation. Hydrolyze at 95°C for 20 minutes.
   - **Serum and Plasma:** Add 1 ml of sample and 1 ml of Alkaline Reagent into a glass tube. Cover with plastic wrap and create small holes for ventilation. Hydrolyze at 95°C for 20 min.
2. **pH Adjustment:** Cool the sample hydrolysate by running the tube under running water. Once cooled, add 1 ml of Acidic Reagent and mix fully. Using pH test paper, measure the pH value of the solution. Adjust to 6.5 by adding Alkaline Reagent or Acidic Reagent drop by drop. Once done, add double-distilled water to make a final volume of 10 ml and mix fully.
3. **Decolorization of Sample Hydrolysate:** Add 1.5 ml of sample hydrolysate into a centrifuge tube. Add 15 mg of Clarifying Reagent and mix fully. Centrifuge at 15000 × g for 10 min. Collect the supernatant for detection.

We recommend carrying 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 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:

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

2. Reagents

Equilibrate all reagents to 25°C before use.

- **Measuring Working Solution:** For each well, prepare 500 µl of Measuring Working Solution by combining equal parts Buffer Solution and Oxidant Solution. For example, add 250 µl Buffer Solution and 250 µl Oxidant Solution. Prepare immediately before carrying out the assay and mix thoroughly.
- **5 µg/ml Standard Solution:** Dilute 0.65 ml of 100 µg/ml Standard Solution in 12.35 ml double-distilled water. Prepare immediately before carrying out the assay and mix thoroughly.

B. Assay Procedure

1. **Standard Dilutions:** Label 8 tubes with 5 µg/ml, 4 µg/ml, 3.5 µg/ml, 3 µg/ml, 2 µg/ml, 1.5 µg/ml, 1 µg/ml, and 0 µg/ml. Prepare Standard Dilutions according to the following dilution scheme:

Concentration (µg/ml)	5	4	3.5	3	2	1.5	1	0
5 µg/ml Standard Solution (ml)	3.0	2.4	2.1	1.8	1.2	0.9	0.6	0
Double-distilled water	0	0.6	0.9	1.2	1.8	2.1	2.4	3.0

2. Operating Steps

Pre-heat the water bath and ensure it has reached a stable temperature before use.

1. Mark microcentrifuge tubes for each standard, sample, and control. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 1 ml of each Standard Dilution to the corresponding standard tube.
3. Add 1 ml of sample to the sample tubes.
4. Add 0.5 ml of Measuring Working Solution to each tube. Mix fully and leave at room temperature for 20 minutes.
5. Add 0.5 ml of Stop Reagent to each tube. Mix fully and leave at room temperature for 5 minutes.
6. Add 0.5 ml of Chromogenic Reagent to each tube. Mix fully and incubate in a water bath at 65°C for 15 minutes.
7. Cool tubes to room temperature with running water.
8. Set the spectrophotometer to zero with double-distilled water and measure the OD value of each tube at 565 nm in a quartz cuvette (1cm optical path).

C. Calculation of Results

Subtract the mean OD value of the blank from all standard readings to give the absolute OD value. The standard curve can be plotted as the absolute OD of each standard solution (*y*) vs. the respective concentration of the standard solution (*x*). A linear fit is recommended for the standard curve (*y* = a*x* + b). The HYP concentration of the samples can be interpolated from the standard curve.

1. Serum and Plasma:

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

2. Tissue:

$$\textbf{HYP content } (\mu g/mL) = \frac{\Delta A - b}{a} \times \frac{V}{m} \times f$$

where:

ΔA	$(\mathrm{OD}_{\mathrm{Sample}} - \mathrm{OD}_{\mathrm{Blank}})$
a	gradient of the standard curve (linear fit)
b	y-intercept of the standard curve (linear fit)
f	dilution factor of the sample before carrying out the assay
V	volume of sample hydrolysate after pH adjustment = 10 ml
V_1	volume of sample (ml)
m	weight of the sample (mg)

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.