

Description

Hydroxyproline is a unique modified amino acid found exclusively in several animal proteins, the most prevalent of which is collagen. Collagen is a key structural protein of the extracellular matrix, common in connective tissues, and is crucial for medical applications such as tissue transplantation and cell-culture scaffolding as well as in the beauty industry. In this kit, hydroxyproline in the sample is first oxidised to a pyrrole ring, which then reacts with a dye reagent to yield a pink product measured at 560 nm. Because hydroxyproline exists almost exclusively in collagen, its content can be used as a proxy for collagen content.

Specifications

Detection Method	Colorimetric; read at 560 nm
Detection Range	0.5 ug/mL to 50 ug/mL hydroxyproline
Sample Type	Biologic and cosmetic samples, serum, cells, urine, tissue lysate, collagen
Species Reactivity	All
Assay Time	Approximately 2 hours
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	Store kit components at 4 degrees C upon receiving.
Shelf Life	12 months after receipt
Shipping	Room Temperature

Kit Components

Component	Quantity	Storage
Reagent A	1 mL	4 degrees C
Reagent B	10 mL	4 degrees C
Oxidation Buffer	10 mL	4 degrees C
Hydroxyproline Standard	200 uL	4 degrees C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0219	100 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. This kit detects free hydroxyproline, so any collagen-containing samples must be hydrolysed prior to assay: combine 50 microlitres of sample with 50 microlitres of 10N NaOH in Eppendorf tubes, heat at 100 degrees C for 1 hour (solid samples may need longer), allow to cool, neutralise with 50 microlitres of 10N HCl, dilute 1:1 with 150 microlitres of deionised water and centrifuge for 2 minutes to collect liquid and pellet debris.
2. For biological fluid samples such as serum, cells, urine and tissue lysate, first centrifuge to remove particulates; a serial dilution may be needed to bring the sample within the detection limit.
3. Prepare a 50 ug/mL Hydroxyproline Standard by mixing 20 microlitres of 1 mg/mL Hydroxyproline Standard with 380 microlitres of distilled water, then prepare standards in 1.5 mL centrifuge tubes as described in the standard preparation table.

4. Transfer 20 microlitres of standards and samples into separate wells of a clear flat-bottom 96-well plate.
5. For each well mix 8 microlitres of Reagent A with 90 microlitres of Oxidation Buffer, add 90 microlitres of this mix to each well, tap the plate to mix and incubate 10 minutes at room temperature.
6. Add 90 microlitres of Reagent B to all wells; the wells will become turbid, so pipette up and down until the turbidity dissipates.
7. Incubate for 90 minutes at 37 degrees C in a plate reader or incubator.
8. Read optical density at 560 nm, using the 90-minute time point for analysis.

Standard Preparation

No	50 ug/mL Standard + H ₂ O	Hydroxyproline (ug/mL)
1	100 uL + 0 uL	50
2	60 uL + 40 uL	30
3	30 uL + 70 uL	15
4	0 uL + 100 uL	0 (Blank)

Calculation

Subtract the blank value (#4) from the standard values and plot the delta-OD against standard concentrations. Determine the slope and calculate the hydroxyproline concentration as: $[\text{Hydroxyproline}] = \frac{(\text{OD}_{\text{sample}} - \text{OD}_{\text{blank}}) / \text{Slope (ug/mL}^{-1})}{n} \times n \text{ (ug/mL)}$, where OD_{sample} and OD_{blank} are the OD readings of the Sample and Blank respectively and n is the sample dilution factor. Note: the dilution factor n for the hydrolysis protocol is 6 (50 uL sample + 50 uL 10N NaOH + 50 uL 10N HCl + 150 uL dH₂O). Conversions: 50 ug/mL equals 5 mg/dL or 50 ppm. Hydroxyproline constitutes 13% of total collagen weight on average, so 1 ug/mL hydroxyproline is equivalent to 1/0.13 or 7.69 ug/mL collagen.

Notes

- This kit detects free hydroxyproline; collagen-containing samples must be hydrolysed prior to assay.
- Briefly centrifuge tubes before opening and equilibrate all components to room temperature prior to assay.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.

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Technical specifications are based on manufacturer documentation and may be updated. Protocols shown are summaries - always follow the instructions supplied with your kit/lot.