Hydroxyproline (HYP) Content Assay Kit (Ehrlich, Micro Method)

Cat. No.: H1515817 | Pack size: 96 tests | Storage: 2-8 °C; protect from light

Overview

Hydroxyproline (HYP) is a non-essential amino acid and a major component of collagen tissue in vivo. With the exception of a small amount in elastin, most hydroxyproline exists in collagen; HYP content is therefore an important indicator of collagen metabolism and tissue fibrosis.

In this assay, free hydroxyproline is released from samples by acid hydrolysis and oxidized to pyrrole by Chloramine-T. Pyrrole reacts with p-dimethylaminobenzaldehyde to form a red compound with characteristic absorbance at 560 nm, allowing quantitative determination of hydroxyproline content.

Key Features

- Colorimetric micro-method for quantitative HYP measurement at 560 nm
- Suitable for animal tissues, bacteria, cultured cells, serum, plasma, and related samples
- Compatible with microplate reader or visible spectrophotometer detection
- Includes ready-to-use reagents and L-hydroxyproline standard
- Recommended for evaluation of collagen metabolism and tissue-fibrosis related research

Contents & Storage

Cat. No.	Component	Size	Storage
H1515817A	Cell Extraction Buffer	120 mL	2-8 °C
H1515817B	Reagent I	8.4 mL	2-8 °C; protect from light
H1515817C	Reagent II	8.4 mL	2-8 °C; protect from light
H1515817D	Standard	0.5 mL	2-8 °C; protect from light

Materials Required But Not Supplied

Item	Recommended Specification	Purpose
Detection instrument	Microplate reader or visible spectrophotometer capable of 560 nm	Absorbance measurement
Water bath / oven / centrifuge	60 °C water bath, 110 °C oven, suitable centrifuge	Hydrolysis, reaction, and clarification
Reaction vessels	96-well plate or micro glass cuvette; 1.5 mL tubes; glass tubes	Reaction and detection
General consumables	Adjustable pipettes and tips	Liquid handling
User-supplied reagents	Deionized water, concentrated HCl, anhydrous ethanol, isopropanol, NaOH	Extraction, dilution, and pH adjustment

Preparation Before Use

1. Equilibrate kit reagents to room temperature before use and protect light-sensitive reagents from light.
2. Prepare a standard curve in every independent experiment. Diluted standard solutions are unstable and should be used within 4 hours.
3. Run a pre-experiment using 2-3 samples expected to have significant differences before formal detection.

Protocol

Reagent Preparation

Reagent Name	Notes	Storage
Tissue Extraction Buffer (self-provided)	6 mol/L hydrochloric acid; concentrated HCl:deionized water = 1:1 (v/v)	Room temperature
Cell Extraction Buffer	Ready-to-use; equilibrate to room temperature before use	2-8 °C

Reagent Name	Notes	Storage
Reagent I	Ready-to-use; equilibrate to room temperature before use	2-8 °C; protect from light
Reagent II	Ready-to-use; equilibrate to room temperature before use	2-8 °C; protect from light
Standard	Ready-to-use 0.5 mg/mL L-hydroxyproline standard; equilibrate before use	2-8 °C; protect from light

Standard Working Solution Preparation

No.	Volume of Stock Standard	Volume of Deionized Water	Final Concentration (μg/mL)
Std.1	24 μL 0.5 mg/mL standard	376 μL	30
Std.2	200 μL of Std.1 (30 μg/mL)	200 μL	15
Std.3	200 μL of Std.2 (15 μg/mL)	200 μL	7.5
Std.4	200 μL of Std.3 (7.5 μg/mL)	200 μL	3.75
Std.5	200 μL of Std.4 (3.75 μg/mL)	200 μL	1.875
Std.6	200 μL of Std.5 (1.875 μg/mL)	200 μL	0.938
Std.7	200 μL of Std.6 (0.938 μg/mL)	200 μL	0.469
Std.8	200 μL of Std.7 (0.469 μg/mL)	200 μL	0.234

Sample Preparation

Fresh samples are recommended. If samples cannot be used immediately, store at -80 °C for up to one month. During detection, control thawing temperature and time and complete thawing within 4 hours at room temperature.

Animal tissue samples: Weigh approximately 0.2 g tissue into a glass test tube and add 2 mL Tissue Extraction Buffer. Hydrolyze at 110 °C for 6-12 h. After cooling, adjust pH to about 7.0 with NaOH, dilute to 4 mL with deionized water, centrifuge at 12,000 x g, 25 °C for 20 min, and collect supernatant for testing.

Bacteria and cultured cells: Collect 5 million bacteria or cells, add 1 mL Cell Extraction Buffer, autoclave at 15 psi for 30 min, and cool naturally. Adjust pH to about 7.0 with concentrated HCl, dilute to 2 mL with deionized water, centrifuge at 12,000 x g, 25 °C for 20 min, and collect supernatant.

Serum free-HYP extraction: Add 0.5 mL absolute ethanol to 0.1 mL serum for protein precipitation. Centrifuge at 8,000 x g, 4 °C for 5 min. Transfer supernatant, evaporate ethanol completely, cool, and dissolve residue in 0.2 mL 50% isopropanol for testing.

Reaction System Setup

Preheat the detector for more than 30 min. Set wavelength to 560 nm and zero the spectrophotometer with deionized water. Set up reactions in 1.5 mL centrifuge tubes as follows:

Reagent	Blank Tube (µL)	Standard Tube (µL)	Sample Tube (µL)
Sample solution	0	0	60
Standard solution	0	60	0
Reagent I	60	60	60
Mix and incubate at room temperature	20 min	20 min	20 min
Reagent II	60	60	60
Deionized water	180	120	120

Measurement

Mix thoroughly, incubate at 60 °C for 20 min, then cool at room temperature for 15 min. Transfer 200 µL of each reaction solution to a 96-well plate or micro cuvette and measure absorbance at 560 nm.

Record ABlank, AStandard, and ASample. Calculate ΔASample = ASample - ABlank and ΔAStandard = AStandard - ABlank. Blank and standard groups generally need to be repeated 1-2 times per experiment.

If ΔASample is below 0.005, increase sample amount appropriately. If ΔASample exceeds the absorbance of the 30 µg/mL standard, dilute the sample with the corresponding extraction buffer and multiply by the dilution factor in final calculation, or reduce initial sample weight/volume.

Result Calculation

Plot the standard curve using standard concentration on the x-axis and ΔAstandard on the y-axis. Substitute ΔAsample into the equation to calculate x (μg/mL).

Based on protein concentration: HYP (μg/mg protein) = x / Cpr.

Based on sample fresh weight: HYP (μg/g fresh weight) = 20x / W.

Based on bacterial or cell count: HYP (μg/10^4 cells) = 10x / n.

Based on sample volume: HYP (μg/mL) = 10x.

Parameters: x = concentration calculated from standard curve; Cpr = protein concentration (mg/mL); W = tissue fresh weight (g); n = number of bacteria or cells in 10^4 units.

Storage & Handling

Store kit components at 2-8 °C and protect light-sensitive reagents from light. Shelf life under recommended storage is 6 months.

Safety & Precautions

1. Biochemical reagents may be irritant or biologically toxic. Wear lab coat, gloves, mask, and hair cover throughout the experiment.
2. Perform operations involving concentrated acid, solvents, or biological samples in a fume hood or biosafety cabinet as appropriate.
3. This product is for scientific research only and is not intended for clinical diagnosis.

Quality Control

QC Item	Method	Acceptable Range
Blank control	Absorbance at 560 nm	Stable low background within laboratory acceptance criteria
Standard curve	Gradient standard solutions	Linear response across the recommended standard range
Sample repeatability	Duplicate or triplicate measurement	Coefficient of variation within validated laboratory limits

Troubleshooting

Issue	Possible Causes	Corrective Action
Low signal	Low HYP content or insufficient sample input	Increase sample dosage or concentrate sample within the validated range
Signal above standard range	High HYP content or insufficient dilution	Dilute the sample and multiply by the dilution factor
Poor standard curve	Old diluted standards or pipetting error	Prepare fresh standards for each experiment and mix thoroughly

Recommended Applications

Quantitative HYP detection · collagen metabolism studies · tissue fibrosis research · animal tissue/cell/serum/plasma assays

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Aladdin Scientific Corporation

14078 Meridian Parkway, Riverside, CA 92518, USA

Phone: 1-833-552-7181

Sales: sales@aladdinsci.com

Customer Service: custserv@aladdinsci.com

EU SALES, LOGISTICS & LOCALIZED SUPPORT

Aladdin Biochem Deutschland GmbH

Westring 2, 33142 Büren, Germany

Phone: +02951 9383958

Support: support.eu@aladdinsci.com

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