QuantiChrom™ Hydroxyproline Assay Kit (DHYP-100)

Quantitative Colorimetric Hydroxyproline/Collagen Determination

DESCRIPTION

HYDROXYPROLINE is a unique modified amino acid that is found exclusively in several animal proteins, the most prevalent of which is collagen. Collagen is a key structural protein of the extracellular matrix and is common in connective tissues due to its incredible strength. Because of its role as a key structural protein in animals collagen is crucial for many medical applications, such as tissue transplantation and scaffolding for complex cell cultures. Collagen is also used in the beauty industry, as it can be supplemented to increase tissue rigidity, resulting in skin that appears more youthful.

BioAssay Systems' Hydroxyproline assay kit delivers a safe, simple, and sensitive means to quantify hydroxyproline in samples. In the first step of this procedure, hydroxyproline in the sample is oxidized to a pyrrole ring. This compound then reacts with a dye reagent to yield a pink product that can be measured at 560 nm. Hydroxyproline exists almost exclusively in collagen, so hydroxyproline content can be used as a proxy for collagen content.

KEY FEATURES

Safe. Our hydroxyproline assay uses an improved perchlorate-free chemistry.

Sensitive and accurate. Uses 20 µL sample. Linear detection range 0.5 µg/mL to 50 µg/mL hydroxyproline in 96-well plate assay.

Convenient. Collagen can be hydrolyzed in Eppendorf tubes, and final incubation can be performed at 37°C.

APPLICATIONS

Direct Assays: hydroxyproline and collagen in biologic and cosmetic samples.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Reagent A: 1 mL **Oxidation Buffer:** 10 mL
Reagent B: 10 mL **Hydroxyproline Standard:** 200 µL

Storage conditions. The kit is shipped at room temperature. Store kit components at 4°C upon receiving. Shelf life: 12 months after receipt.

Precautions: reagents are for research use only. Briefly centrifuge tubes before opening. Equilibrate all components to room temperature prior to assay. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Collagen Sample Hydrolysis: This kit detects free hydroxyproline, so any samples containing collagen must be hydrolyzed prior to assay. To hydrolyze samples combine 50 µL sample with 50 µL 10N NaOH in Eppendorf tubes. Heat tubes at 100ºC for 1 hour *(note: solid samples may need longer hydrolysis)*. Allow tubes to cool, then neutralize tubes with 50 µL 10N HCl. Dilute samples 1:1 with 150 µL deionized water. Centrifuge tubes for 2 minutes to collect all liquid in the tube and to pellet debris.

Biological fluid samples (e.g. serum, cells, urine, tissue lysate) first centrifuge to remove any particulates. A serial dilution may be needed to ensure the sample falls within the kit's detection limit.

Procedure using 96-well plate

1. *Standards Prep* Prepare 50 µg/mL Hydroxyproline Standard by mixing 20 µL 1 mg/mL Hydroxyproline Standard and 380 µL dH_2O. Next prepare standards in 1.5-mL centrifuge tubes as described in the table below.

No	50 µg/mL Standard + H_2O	Hydroxyproline (µg/mL)
1	100 µL + 0 µL	50
2	60 µL + 40 µL	30
3	30 µL + 70 µL	15
4	0 µL + 100 µL	0 (Blank)

2. Transfer 20 µL standards and samples into separate wells of a clear, flat-bottom 96-well plate.
3. For each well mix 8 µL Reagent A with 90 µL Oxidation Buffer. Add 90 µL of this mix to each well. Tap plate to mix and incubate 10 minutes at room temperature.
4. Add 90 µL Reagent B to all wells. When Reagent B is added the wells will become turbid, you must pipette up and down until the turbidity dissipates.
5. Incubate for 90 minutes at 37°C in plate reader or incubator.
6. Read ODs at λ = 560 nm. Use OD at 90 minute time point for analysis.

CALCULATION

Subtract the blank value (#4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the hydroxyproline concentration of the sample.

$$[\text{Hydroxyproline}] = \frac{OD_{sample} - OD_{blank}}{\text{Slope } (\mu g/mL^{-1})} \times n \ (\mu g/mL)$$

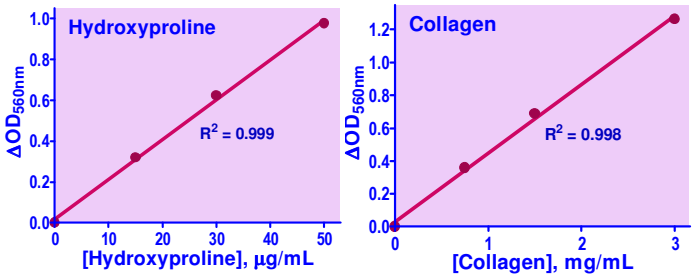
where OD_{sample} and OD_{blank} are OD readings of the Sample and Blank, respectively. *n* is the sample dilution factor. *Note: The dilution factor n for the hydrolysis protocol is 6 (50 µL sample + 50 µL 10N NaOH + 50 µL 10N HCl + 150 µL dH_2O).*

Conversions: 50 µg/mL equals 5 mg/dL, or 50 ppm. Hydroxyproline constitutes 13% of total collagen weight on average. 1 µg/mL Hydroxyproline is equivalent to 1/0.13 or 7.69 µg/mL collagen.

Examples: a bovine skin collagen solution purchased from Sigma-Aldrich (cat# C4243-20mL) was digested as described above and its collagen content was determined to be 3.3 ± 0.2 mg/mL.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, 10 N NaOH and HCl, clear flat-bottom 96-well plates, plate reader, heat block or hot water bath, incubator.



Hydroxyproline and Collagen Assay in 96-Well Plate

LITERATURE

1. Parenteau-Bareil, R., Gauvin, R., & Berthod, F. (2010). Collagen-based biomaterials for tissue engineering applications. *Materials*, *3*(3), 1863-1887.
2. Chattopadhyay, S., & Raines, R. T. (2014). Collagen-Based Biomaterials for Wound Healing. *Biopolymers*, *101*(8), 821.
3. Lee, A., et al (2019). 3D bioprinting of collagen to rebuild components of the human heart. *Science*, *365*(6452), 482-487.

Related Kits

Collagen Assay Kit (Cat# ECOL-100)
L-Amino Acid Assay Kit (Cat# EDAA-100)
D-Amino Acid Assay Kit (Cat# ELAA-100)
Phenylalanine Assay Kit (Cat# EPHE-100)
Tryptophan Assay Kit (Cat# ETRP-100)
Alanine Assay Kit (Cat# EALA-100)
Aspartate Assay Kit (Cat# EASP-100)
Glutamine Assay Kit (Cat# EGLN-100)
Glutamate Assay Kit (Cat# EGLT-100)
Protein Assay Kit (Cat# QCPR-500)