Hydroxyproline Colorimetric Assay Kit (BN00785)

(Catalog # BN00785; 100 assays; Store at +4°C)

I. Introduction:

Hydroxyproline (4-hydroxyproline, Hyp) is a common nonproteinogenic amino acid. It is found only in collagen and elastin in mammals but exists in a number of other proteins in plants. Hydroxyproline is formed only as a post-translational modification in the peptide chain and proline hydroxylase does not hydroxylate free proline. Hydroxyproline in tissue hydrolysates is a direct measure of the amount of collagen or gelatin present. A variety of disease states are believed to affect collagen turnover and can cause elevated serum or urine hydroxyproline. Such conditions range from neoplastic, inflammatory, renal or bone disease to endocrine and autoimmune disorders. Assay Genie's Hydroxyproline Assay Kit is designed to measure hydroxyproline in tissue or protein/peptide hydrolysates. It can be used to measure hydroxyproline from other biological samples such as serum or urine if they have undergone a prior purification process. It is an easy, convenient method which results in a chromogen with an absorbance maximum at 560 nm. The assay is useful over the range of 0.1-2 µg.

II. Application:

- Measurement of hydroxyproline in various tissues or protein/peptide hydrolysates, serum & urine

III. Sample Type:

- Animal tissues
- protein/peptide hydrolysates
- serum
- urine

IV. Kit Contents:

Components	BN00785	Cap Code	Part Number
Oxidation Buffer	10 ml	WM	BN00785-1
Chloramine T Concentrate	0.6 ml	Red	BN00785-2
Perchloric acid/Isopropanol Solution	5 ml	NM	BN00785-3
DMAB Concentrate (in DMSO)	5 ml	Amber	BN00785-4
Hydroxyproline Standard (1 mg/ml)	0.1 ml	Yellow	BN00785-5

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer
- For hydrolysis: Polypropylene Vials and Screw Caps

VI. Storage and Handling:

Store kit at +4°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

VII. Reagent Preparation and Storage Conditions:

- **Chloramine T Reagent:** For each well to be analyzed, add 6 µl of Chloramine T Concentrate to 94 µl of Oxidation Buffer and mix well.
- **DMAB Reagent**: For each well to be analyzed, add 50 µl of DMAB Concentrate to 50 µl of Perchloric acid/Isopropanol Solution and mix well. Keep on ice, protected from light.

  Note: The reagent concentrates are stable as supplied. Once the concentrates have been diluted to working concentration, they are only good for 2-3 hours so only make as much reagent as necessary for the number of samples and standards to be quantified.

VIII. Hydroxyproline Assay Protocol:

1. **Sample Preparation:** Tissue or protein/peptide samples such as lung tissue should be homogenized in dH_2O, using 100 µl H_2O for every 10 mg of tissue. To a 100 µl of sample homogenate, add 100 µl concentrated HCl (~12N, not provided) in a pressure-tight, teflon capped vial and hydrolyze at 120°C for 3 hours. In case of urine, hydrolyze urine samples with equal volumes of concentrated HCl (~12 N; i.e. 100 µl Urine + 100 µl HCl) in a pressure-tight, teflon capped vial. Hydrolyze at 120°C for 3 hrs (**See note c**). Clarify urine samples with activated charcoal by adding 4 mg of activated charcoal. Vortex and centrifuge at 10000 x g for 3 min. to remove precipitate & activated charcoal. Repeat if needed. Transfer 10 µl of each hydrolyzed sample to a 96-well plate and evaporate to dryness under vacuum.

   Notes:

   a. For unknown samples, we suggest performing a pilot experiment & testing different sample dilutions to ensure the readings are within the Standard Curve range.

   b. Endogenous compounds may interfere with the reaction. To ensure accurate determination of Hydroxyproline in the test samples, we recommend spiking samples with a known amount of Standard (0.4 µg).

   c. For sample hydrolysis, polypropylene vials yield best results. We recommend Assay Genie's Polypropylene Vials and Caps

2. **Standard Curve Preparation:** Dilute the Hydroxyproline Standard to 0.1 mg/ml by adding 10 µl of the 1 mg/ml Standard to 90 µl of dH_2O, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells to generate 0.2, 0.4, 0.6, 0.8 & 1 µg/well Hydroxyproline Standard.
3. **Reaction:** Add 100 µl of the Chloramine T reagent to each sample and standard and incubate at room temperature for 5 min. Add 100 µl of the DMAB reagent to each well and incubate for 90 min. at 60°C.
4. **Measurement:** Measure absorbance at 560 nm in a microplate reader.

5. **Calculation:** Correct background by subtracting the value derived from the 0 Hydroxyproline Standard from all readings (The background reading can be significant and must be subtracted). Plot the Standard curve. Apply the sample readings to the standard curve to get the hydroxyproline amount in the reaction wells (B).

Sample Hyp concentration (C) = B/V X D µg/µl

Where: **B** is the amount of Hydroxyproline from Standard Curve (µg)
V is the sample volume added into the reaction well (µl)
D is the sample dilution factor

Note: For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading.

For spiked samples, Hyp amount in sample well (B) = $\left(\frac{(\mathrm{OD}_{\text{sample (corrected)}})}{(\mathrm{OD}_{\text{sample + Hyp Std(corrected)}})-(\mathrm{OD}_{\text{sample(corrected)}})}\right) * Hyp\ Spike\ (\mu g)$

Hydroxyproline MW: 131.13 g/mol

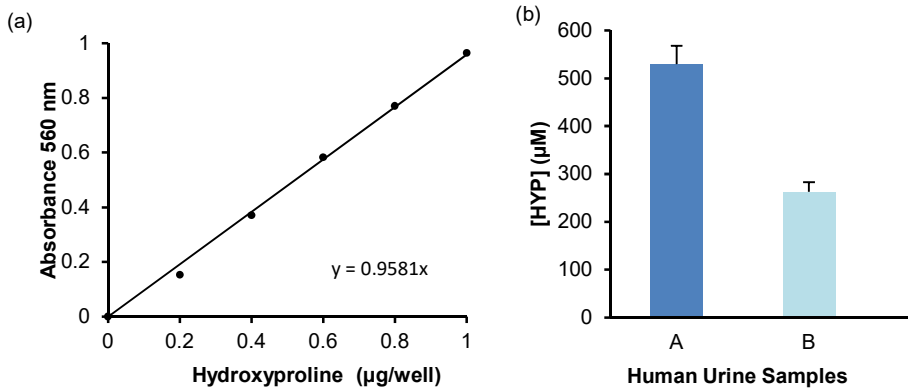

Figure. a.) Hydroxyproline Standard Curve. b.)Determination of hydroxyproline (Hyp) concentration in human urine. Briefly, samples were hydrolyzed with 12 N HCl for 3 hrs at 120°C and clarified using activated charcoal. Activated charcoal and precipitates were removed by centrifugation (10000 x g, 3 min.). 10 µl of samples were spiked with 0.4 µg Hydroxyproline Standard. Samples were collected during different diet conditions. For an example, sample A was collected after strenuous exercise followed by fasting for 12 hrs. Sample B was collected 7 hours after sample A following normal uptake of food (900 calories). Assays were performed according to the kit protocol.

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