Product Information

Hydroxyproline Colorimetric Assay Kit

I. Kit Contents:

Components	K2083-100 100 assays	Cap Color	Part Number
Oxidation Buffer	10 ml	WM	K2083-C-1
Chloramine T Concentrate	0.6 ml	Red	K2083-C-2
Perchloric acid/Isopropanol Solution	5 ml	NM	K2083-C-3
DMAB Concentrate (in DMSO)	5 ml	Amber	K2083-C-4
Hydroxyproline Standard (1 mg/ml)	0.1 ml	Yellow	K2083-C-5

II. Introduction:

Hydroxyproline is a common nonproteinogenic amino acid. In mammals, hydroxyproline is found only in elastin and collagen. However, it exists in a variety of other proteins in plants. Hydroxyproline is formed by proline hydroxylase in the peptide chain as a post-translational modification and can not be formed from free proline. A variety of diseases such as neoplastic, renal or bone disease, inflammatory, endocrine and autoimmune disorders affect collagen turnover and cause elevated urine and serum hydroxyproline. Hydroxyproline hydrolysates in tissue can be used for the measurement of the amount of collagen or gelatin present.

The Hydroxyproline Colorimetric Assay Kit provides a sensitive, simple and convenient way for detection of hydroxyproline in tissue or protein/peptide hydrolysates based on colorimetric method. The assay can also be used for measurement of hydroxyproline content in other biological samples such as serum or urine with a prior purification process. The result can be easily qualified using a microtiter plate reader or a spectrophotometer at 560 nm. The assay is useful over the range of 0.1 - 2 μg.

III. Application:

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

IV. Sample Type:

Animal tissues.

protein/peptide hydrolysates.

serum.

urine.

V. User Supplied Reagents and Equipment:

96-well clear plate with flat bottom.

Multi-well spectrophotometer.

VI. Storage and Handling:

Store kit at +4 ℃ 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 dH2O, using 100 µl H2O 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 ℃ 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 ℃ for 3 hrs. 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).

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 ℃.

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 × 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) = (OD sample (corrected))/ [OD sample + Hyp Std(corrected)× OD sample (corrected))]* Hyp Std(corrected) (µg).

Hydroxyproline MW: 131.13 g/mol.

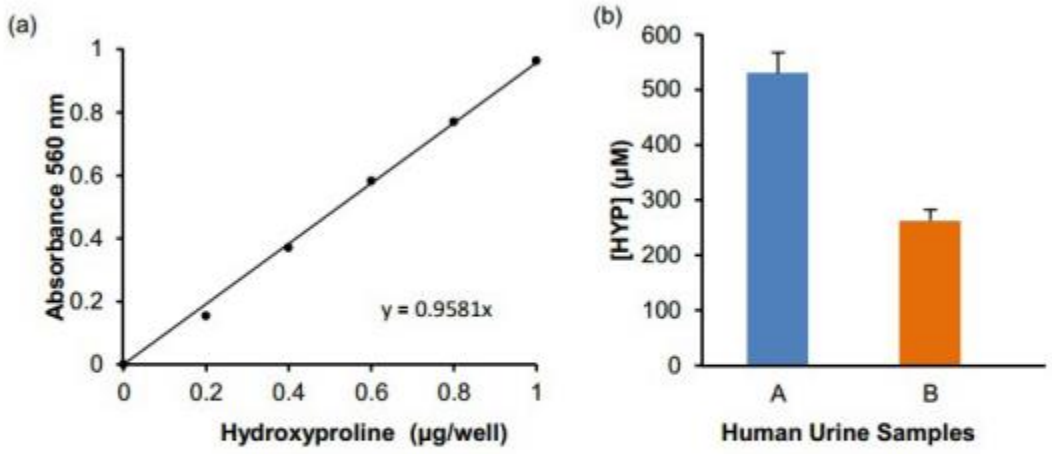

Figure. a.) Hydroxyproline Standard Curve. b.)Determination of hydroxyproline (Hyp) concentration in human urine. Briefly, samples were hydrolyzed with 12 N HCl for 3 hrs at 120°C and clarified using activated charcoal. Activated charcoal and precipitates were removed by centrifugation (10000 x g, 3 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.

Frequently Asked Questions

1. Should the Hydroxyproline Standard also go through the drying process as hydrolzed samples? If it is possible, could you tell me the reasons why the hydrolyzing process is required?

The samples are being digested/hydrolyzed with acid to calculate the hydroxyproline from the collagen. The standard is hydroxyproline so it does not need the HCL/cooking treatment. In addition, the standard is not vacuum dried because it does not contain HCl-the samples are dried to remove the HCl before proceeding with the other reagents.

2. In the manual it says to dry the plate under vacuum. Since I don't have the equipment readily available for this, I was wondering if the plate could be dried in an oven at, for example, 37℃ or just to air at room temperature

You can dry the plate in an oven at 60℃ make sure to get rid of the HCl. Air drying will take a very long time and is not recommended.

6. Is it possible to keep the hydrolyzed lysate or the dried 96-well plate for continuation at a later time? If possible, what are the storage conditions?

Yes, you can store either / both the hydrolyzed sups and the dried plate at -80℃ for later use. Please aliquot the lysate and seal the plate tight before storing.

7. Is it is acceptable to reduce the reaction time in sample preparation part, 120℃, 3 hours? If yes, how long should be done at least?

The hydrolysis will not be completed efficiently if done for lesser than 3 hrs. If your concern for the time is that you would not be able to finish the whole expt the same day, you can always hydrolyze O/N as well and continue the next day, or hydrolyze for 3 hrs and freeze the hydrolyzed sup at 80℃ in aliquots for use the next day.

8. Can this kit be used with samples like bacteria, plants, drosophila, yeast etc?

We have optimized the kit with mammalian samples. However, theoretically these kits should work with samples from multiple species/sources. Since the optimal conditions depend on the sample type, the protocol has to be be adapted to fit the samples for efficient results. Please refer to this kit's citations to see what kind of samples have been used with this kit other than mammalian samples.

9. Can we use frozen samples with this assay?

Fresh samples are always preferred over frozen samples. However, frozen samples can also be used, provided, they were frozen right after isolation, were not freeze thawed multiple time (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods.

10. Can we use a different wavelength than recommended for the final analysis?

It is always recommended to use the exact recommended wavelength for the most efficient results. However, most plate readers have flexibility in their band width of detection in increments of +/- 10 nm. Depending on this flexibility range, you can deviate from the recommended wavelengths within limits.

11. What is the exact volume of sample required for this assay?

There is no specific volume we can recommend for the amount any sample to be used since it is completely sample concentration and quality based. You have to do a pilot expt with multiple sample volumes to determine the optimal volume which gives a reading within the linear range of the standard curve. Please refer to the citations for this product to see what other clients have used with similar sample types.

12. What is the shelf life of this kit?

This kit is good for 12 months from the date of shipment in the unopened form when stored at the appropriate temperature and appropriate conditions. After opening and reconstitution, some of the components in this kit are good for 2 months at -20℃. Please refer to the datasheet for storage information and shelf life of each of the components.

13. Why are my standard curve values lower than those shown on the datasheet?

There are multiple factors which influence the signals like the incubation times, room temperature, handling etc. In general, to increase the value of the standards, you can increase the incubation time. As long as the standard curve is linear, it should be fine to use, since all of your samples will also be measured under the same conditions on this curve.

14. How do I normalize my samples against protein concentration

You can use a protein quantitation assay on the supernatants you get from cell/tissue lysates or with any other liquid sample in the assay buffer.

15. Should I make a standard curve for every expt I do, or is one curve/kit enough?

Yes, I would strongly recommend you to do the standards every time you do the expt. There is always a chance that something was done differently that day and we do not want any conditions to differ between standards and samples.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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