Hydroxyproline Assay Kit

(Catalog #K555-100; 100 assays; Store kit at +4°C)

I. Introduction:

Hydroxyproline (4-hydroxyproline) 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. AMSBIO'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. Kit Contents:

Components	100 Assays	Cap Color	Part Number
Oxidation Buffer	10 ml	WM	K555-100-1
Chloramine T Concentrate	0.6 ml	Red	K555-100-2
Perchloric acid/Isopropanol Solution	5 ml	NM	K555-100-3
DMAB Concentrate (in DMSO)	5 ml	Amber	K555-100-4
Hydroxyproline Standard (1 mg/ml)	0.1 ml	Yellow	K555-100-5

III. General Consideration, Storage and Handling:

Store the kit at +4°C and protect from light. Please read the entire protocol before performing the assay. 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 enough each reagent as necessary for the number of samples and standards to be quantified.

IV. Reagent preparation:

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.

V. Assay Protocol:

1. **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. Adjust volume to 50 µl/well with dH_2O to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 µg/well of the Hydroxyproline Standard.

2. **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 sample of homogenate, add 100 µl concentrated HCl (~12N, not provided) in a pressure-tight, teflon capped vial and hydrolyze at 120°C for 3 hours.

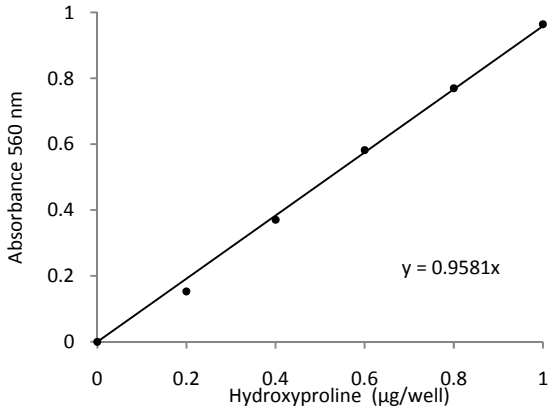
3. **Reaction:** Transfer 10 µl of each hydrolyzed sample to a 96 well plate and evaporate to dryness under vacuum. Add100 µl of the Chloramine T reagent to each sample and standard and incubate at room temperature for 5 minutes. Add 100 µl of the DMAB reagent to each well and incubate for 90 minutes at 60°C.

4. **Read:** Measure the 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 in the reaction wells (Sa). Sample hydroxyproline concentrations:

$$C = S_a/S_v \quad \mu g/\mu l$$

Where S_a is the sample amount (in µg) from standard curve
S_v is the sample volume (µl) added into the wells
Hydroxyproline MW: 131.13 g/mol



Hydroxyproline Standard Cure performed following the kit protocol.

VI. Related Products:

NAD(P)/NAD(P)H Quantification Kit
Ascorbic Acid Quantification Kit
Glucose Assay Kit
Uric Acid Assay Kit
Pyruvate Assay Kit
Triglyceride Assay Kit
Choline/Acetylcholine Quantification Kit
Antioxidant Capacity (TAC) Assay Kit
L-amino Acid Assay Kit
Ethanol Assay Kit
ADP/ATP Ratio Assay Kit
Glutathione Detection Kits
Fatty Acid Assay Kit
Alanine Assay Kit
Lactate Assay Kit/ II
Phosphate Assay Kit
Hemin Assay Kit
Glycogen Assay Kit
Nitric Oxide Assay Kits
Urea Assay Kit

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GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note# The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		