

Hydroxyproline Assay Kit

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



DATA SHEET

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. BioVision'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 dH₂O, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells.

2. 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 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:

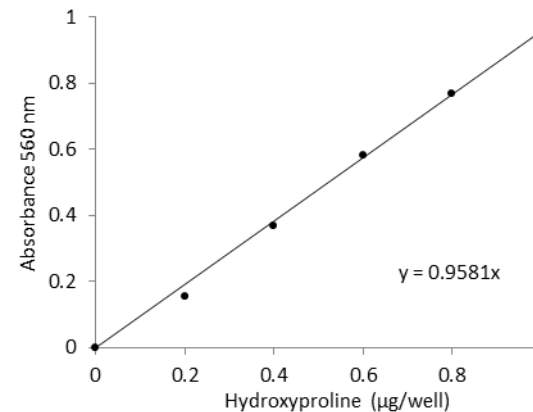
Transfer 10 µl of each hydrolyzed sample to a 96 well plate and evaporate to dryness under vacuum. Add 100 µl of the Chloramine T reagent to each sample and standard and incubate at room temperature for 5 minutes. Add 100 µl of the DMAB reagent to each well and incubate for 90 minutes at 60°C.

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 (S_a). Sample hydroxyproline concentrations:

$$C = S_a/S_v \quad \mu\text{g}/\mu\text{l}$$

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 Curve performed following the kit protocol.

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

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

| Problems | Cause | Solution |
|--|---|--|
| Assay not working | <ul style="list-style-type: none"> • Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate | <ul style="list-style-type: none"> • Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates |
| Samples with erratic readings | <ul style="list-style-type: none"> • Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples | <ul style="list-style-type: none"> • Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use |
| Lower/ Higher readings in Samples and Standards | <ul style="list-style-type: none"> • Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used | <ul style="list-style-type: none"> • Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly |
| Readings do not follow a linear pattern for Standard curve | <ul style="list-style-type: none"> • Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots | <ul style="list-style-type: none"> • Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit |
| Unanticipated results | <ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range | <ul style="list-style-type: none"> • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • 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.