Hydroxyproline Assay Kit (Colorimetric) ab222941

Overview

Product name: Hydroxyproline Assay Kit (Colorimetric)
Detection method: Colorimetric
Sample type: Urine, Serum, Other biological fluids, Tissue Homogenate
Assay type: Quantitative
Range: 0.1 µg - 1 µg
Assay time: 3h 00m
Species reactivity: Reacts with: Other species, Mammals

Product overview

Hydroxyproline Assay Kit (Colorimetric) ab222941 provides a quick and convenient method to quantify hydroxyproline in tissue lysates and biological fluids such as urine and serum.

The classical hydroxyproline assay protocol is based on the oxidation of hydroxyproline to a pyrrole intermediate followed by reaction with Ehrlich’s reagent dissolved in concentrated perchloric acid. Perchloric acid is a hazardous material that is both toxic and highly reactive, requiring special handling and waste-disposal protocols.

This hydroxyproline assay protocol employs a proprietary acidic developer solution to accurately measure hydroxyproline in hydrolysates without the use of hazardous perchlorates. It is a quick and convenient protocol where hydroxyproline gets oxidized to form a reaction intermediate, which further in reaction forms brightly-colored chromophore that can be easily detected at OD 560 nm.

The assay can detect as low as 0.05 µg hydroxyproline/well.

Notes

Hydroxyproline assay protocol summary:
- add 10 N concentrated NaOH to samples and hydrolyze at 120°C for 1 hr
- cool on ice
- neutralize with 10 N concentrated HCl, centrifuge and collect supernatant
- add samples and standards to wells
- evaporate wells to dryness by heating at 65°C
- add oxidation reagent mix to dissolve crystalline residue, and incubate at room temp for 20 min
- add developer and incubate at 37°C for 5 min
- add DMAB concentrate and incubate for 45 min at 65°C
- analyze with microplate reader

Platform

Microplate reader
Storage instructions

Store at -20°C. Please refer to protocols.

<table>
<thead>
<tr>
<th>Components</th>
<th>100 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloramine T Concentrate</td>
<td>1 x 600µl</td>
</tr>
<tr>
<td>Developer</td>
<td>1 x 5ml</td>
</tr>
<tr>
<td>DMAB Concentrate</td>
<td>1 x 5ml</td>
</tr>
<tr>
<td>Hyp Standard</td>
<td>1 x 100µl</td>
</tr>
<tr>
<td>Oxidation Buffer</td>
<td>1 x 10ml</td>
</tr>
<tr>
<td>Plate sealer</td>
<td>1 unit</td>
</tr>
</tbody>
</table>

Relevance

Hydroxyproline, a non-essential amino acid derived from proline, with no known therapeutic use. Hydroxyproline is used as a major component of structural proteins such as collagen, connective tissues, plant cell walls, tendons and ligaments and provides skin elasticity. Vitamin C is required for the conversion process from proline to hydroxyproline, a deficiency in vitamin C can lead to defects in collagen synthesis, thus, resulting in easy bruising, internal bleeding, breakdown of connective tissue of the ligaments and tendons, and increased risk to blood vessel damage. An unusual feature of this amino acid is that, it is not incorporated into collagen during biosynthesis at the ribosomal level, but is formed from proline by a posttranslational modification by an enzymatic hydroxylation reaction.

Images

Hydroxyproline Assay Kit (Colorimetric) (ab222941)
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Typical Hydroxyproline standard calibration curve.

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