Proline Assay Kit

Catalog No.: abx298884

Size: 100 tests (96 samples)

Detection Range: 0.17 µg/ml – 35 µg/ml

Sensitivity: 0.17 µg/ml

Storage: Store all components at 2 – 8°C for up to 6 months.

Application: For detection and quantification of Proline concentration in plant tissue and honey samples.

Introduction

Proline is an aliphatic amino acid used in the biosynthesis of proteins. The side chain from the alpha carbon connects to the nitrogen forming a pyrrolidine loop. In humans, it is a non-essential amino acid as it can be biosynthesized from L-glutamate.

Abbexa's Proline Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Proline concentration. The absorbance should be measured at 520 nm. The intensity of the absorbance is proportional to the concentration of the Proline, which can then be calculated.

Kit components

1. Extracting solution: 60 ml × 5 vials
2. Ninhydrin: 1 vial
3. Acid reagent: 50 ml × 2 vials
4. Proline standard (100 µg/ml): 1vial

Materials Required But Not Provided

1. Spectrophotometer (520 nm)
2. Double distilled water
3. Acetic acid
4. Methylbenzene
5. Absolute ethyl alcohol
6. Pipette and pipette tips
7. Vials/tubes
8. Sonicating water bath
9. Centrifuge
10. Vortex mixer

For Reference Only

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Honey:** Weigh the honey sample. For each 1 g of sample, add 10 ml Extracting solution. Incubate in water bath at 100°C for 15 minutes. Mix fully and allow to cool at room temperature. Centrifuge the solution at 10,000 x g at 4°C for 15 min. Collect the supernatant and assay immediately.
- **Tissue Homogenates:** Weigh the tissue homogenate. For each 1 g of homogenate, add 10 ml Extracting solution. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 10,000 x g at 4°C for 15 min. Collect the supernatant and assay immediately.

Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay procedure.

The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Green Pepper Tissue Homogenate	1
Carrot Tissue Homogenate	1
Lettuce Leaf Tissue Homogenate	1
Honey	1
Epipremnum aureum Tissue Homogenate	1
Cabbage Tissue Homogenate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

For Reference Only

2. Reagents

- **Chromogenic agent:** Mix a vial of Ninhydrin, Acetic acid, and Acid reagent at a ratio of 1:24:16. Heat at < 70°C to dissolve completely. Prepare immediately before carrying out the assay and store in dark after cooling.
- **10 µg/ml standard working solution**: Dilute the 100 µg/ml proline standard 1:9 with Extracting solution. Prepare immediately before carrying out the assay and mix fully.

B. Assay Procedure

1. Set the Blank, Standard and Sample tubes.
2. Add 2 ml of Extracting solution to Blank tube.
3. Add 2 ml of prepared standard to Standard tube.
4. Add 2 ml of sample to the Sample tube.
5. Add 2 ml of Acetic acid, 2 ml of Chromogenic agent to each tube and mix fully with vortex mixer.
6. Fasten the mouth of tubes with plastic film and make a hole with a needle. Incubate in a water bath at 100°C for 30 minutes.
7. Allow the tubes to cool under running water.
8. Add 4 ml of methylbenzene to each tube.
9. Mix fully and allow all the tubes to stand at room temperature for 10 minutes.
10. Take 3 ml of supernatant and centrifuge at 2325 × g for 10 minutes.
11. Take the upper red methylbenzene layer and set the spectrophotometer to zero with methylbenzene.
12. Measure the OD of each tube with a spectrophotometer at 520 nm.

C. Calculation of Results

Proline concentration in plant tissue and honey samples:

$$\text{Pro concentration } (\mu g/g) = \frac{\Delta A_1}{\Delta A_2} \times \frac{c \times V}{W \times f}$$

where:

ΔA_1	OD value of the sample ($OD_{Sample} - OD_{Blank}$)
ΔA_2	OD value of the sample ($OD_{Standard} - OD_{Blank}$)
c	concentration of standard (10 µg/ml)
f	dilution factor of the sample before carrying out the assay
V	volume of sample reagent 1 for sample preparation
W	weight of sample

For Reference Only