Phenylalnine Ammonia Lyase (PAL) Assay Kit

Catalog No.: abx097984

Size: 100 tests

Detection Range: 0.78 U/g – 156 U/g

Sensitivity: 0.78 U/g

Storage: Store all components at 4°C.

Application: For detection and quantification of Phenylalnine Ammonia Lyase activity in plant tissues.

Introduction

Phenylalanine ammonia lysate (PAL) catalyses L-phenylalanine to produce trans-cinnamic acid, which has an absorbance maximum at 290 nm.

Phenylalnine Ammonia Lyase (PAL) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Phenylalnine Ammonia Lyase activity. The absorbance should be measured at 290 nm. The intensity of the color is proportional to the activity of Phenylalnine Ammonia Lyase, which can then be calculated.

Kit components

1. Extraction Buffer: 60 ml
2. Buffer Solution: 2 × 50 ml
3. Substrate: 4 vials
4. Stop Solution: 6 ml

Materials Required But Not Provided

1. Spectrophotometer (290 nm)
2. Double distilled water
3. PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Vials/tubes
6. Incubator (37 °C)
7. Centrifuge
8. Dounce homogenizer
9. Vortex mixer

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.

- **Tissue Homogenates:** Wash approximately 20 mg of tissue in cold PBS (0.01 M, pH 7.4), and homogenize 20 mg tissue in 180 µl Extraction Buffer using a Dounce homogenizer or equivalent at 4 °C. Centrifuge the homogenate at 10,000 x g at 4°C for 10 min. If any particulate remains, centrifuge again. Collect the supernatant and assay immediately.

Sample	Dilution factor
10 % Epipremnum aureum tissue homogenate	1
10% Carrot tissue homogenate	1
10% Green pepper tissue homogenate	1
10% Corn grain tissue homogenate	1

We recommend carrying out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Samples should be diluted using Extraction Buffer.

2. Reagents

Bring all reagents to room temperature prior to use.

- **Substrate working solution:** Dissolve a vial of substrate in 6 ml double distilled water. The reconstituted substrate may be stored at 4 °C for up to a month.

B. Assay Procedure

1. Set control and sample tubes, and label accordingly.
2. Add 800 µl of buffer solution to the control tube, and 780 µl of buffer solution to the sample tubes.
3. Add 20 µl of sample to the sample tubes.
4. Add 200 µl of substrate working solution to the sample and control tubes.
5. Mix fully with the vortex mixer, and incubate at 37 °C for 30 minutes accurately.
6. Add 40 µl of stop solution to all tubes and mix fully with the vortex mixer.
7. Allow all tubes to stand for 5 minutes. Meanwhile, calibrate the spectrophotometer to zero with double distilled water (1 cm optical path, 290 nm).
8. Measure the absorbance of each tube at 290 nm.

C. Calculation of Results

One unit of PAL activity is defined as the quantity of PAL in 1 g of tissue, 1 ml reaction system, that causes a 0.1 OD value change per minute at 37°C.

$$\text{PAL (U/g tissue)} = \frac{\Delta A_{290} \times V_2}{0.1 \times t} \times \frac{V_3}{m \times V_1} \times f$$

where:

ΔA_{290}	OD value of the sample ($\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}$)
m	mass of sample
V_1	volume of sample (0.02 ml)
V_2	total volume of reaction system (1.04 ml)
V_3	volume of added Extraction buffer
t	reaction time (30 min)
f	sample dilution factor prior to assay

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.