Phenylalanine Ammonia-Lyase Assay Kit

Catalog No.: abx097984

Size: 100 Assays

Storage: Store all components at 4°C.

Application: For quantitative detection of Phenylalanine Ammonia-Lyase activity in tissue homogenates and cell lysates.

Introduction: Phenylalanine Ammonia-Lyase (PAL) is an enzyme found in plants and some micro-organisms. It catalyzes the first step of the biosynthesis of phenylpropanoids: a reaction converting L-phenylalanine to ammonia and trans-cinnamic acid. Its activity is induced in response to various stimuli, including tissue damage, light, temperature, and hormones.

Abbexa's Phenylalanine Ammonia-Lyase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Phenylalanine Ammonia-Lyase activity. Trans-cinnamic acid, one of the reaction products, has an absorbance maxima at 290 nm. The concentration of trans-cinnamic acid can be calculated by measuring the absorbance at 290 nm, from which the enzyme activity can be calculated.

Kit components

1. 96 well microplate
2. Assay Buffer: 4 × 30 ml
3. Reaction Buffer: 30 ml
4. Substrate: 1 vial
5. Stop Solution: 4 ml
6. Plate sealer: 3

Materials Required But Not Provided

1. Microplate reader (290 nm) and incubator
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Timer
6. Ice
7. Sonicator
8. Mortar

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Substrate Solution**
  Add 10 ml of distilled water into the Substrate vial and mix thoroughly to prepare the Substrate Solution. Ensure that the Substrate has completely dissolved prior to use. The Substrate Solution can be stored at 4°C after reconstitution.

2. Sample

- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer on ice for 1 hour. Centrifuge at 8000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

1. Set the sample and control wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
2. Add 10 µl of sample to the sample wells.
3. Add 120 µl of Reaction Buffer to all wells.
4. Add 50 µl of Substrate Solution to all wells.
5. Tap the plate gently to mix. Incubate at 30°C for 30 minutes.
6. Add 20 µl of Stop Solution to all wells.
7. Add 10 µl of sample to the control wells.
8. Tap the plate gently to mix. Read and record absorbance at 290 nm.

C. Calculations

One Unit (U) of Phenylalanine Ammonia-Lyase activity is defined as the quantity of enzyme required to change the O.D. of the reaction system by 0.01 per minute.

Phenylalanine Ammonia-Lyase activity per mg of protein:

$$\textbf{Acetolactate Synthase}\ (\mathbf{U/mg}) = \frac{\mathbf{V_{Total}}}{\mathbf{V_{Sample}} \times \mathbf{C_{Protein}} \times \mathbf{T}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Control}}}{\mathbf{0.01}} = \frac{\mathbf{66.7}}{\mathbf{C_{Protein}}} \times (\mathbf{OD_{Sample}} - \mathbf{OD_{Control}})$$

Phenylalanine Ammonia-Lyase activity per g of sample:

$$\textbf{Acetolactate Synthase}\ (\mathbf{U/g}) = \frac{\mathbf{V_{Total}} \times \mathbf{V_{Assay}}}{\mathbf{V_{Sample}} \times \mathbf{W} \times \mathbf{T}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Control}}}{\mathbf{0.01}} = \frac{\mathbf{66.7}}{\mathbf{W}} \times (\mathbf{OD_{Sample}} - \mathbf{OD_{Control}})$$

where:

$\mathbf{C_{Protein}}$	Concentration of protein (in mg/ml)
$\mathbf{W}$	Weight of the sample (in g)
$\mathbf{V_{Assay}}$	Volume of Assay Buffer (1 ml)
$\mathbf{V_{Sample}}$	Volume of sample (0.01 ml)
$\mathbf{V_{Total}}$	Total volume of the reaction (0.2 ml)
$\mathbf{T}$	Reaction time (30 min)