PRODUCT INFORMATION & MANUAL

Phenylalanine Ammonia Lyase/PAL Activity Assay Kit (Colorimetric)

NBP3-25907

For research use only.

Not for diagnostic or therapeutic procedures.

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Novus kits are guaranteed for 6 months from date of receipt

Phenylalanine Ammonia Lyase/PAL Activity Assay Kit (Colorimetric)

Catalog No: NBP3-25907

Method: Colorimetric method

Specification: 100Assays (Can detect 98 samples without duplication)

Measuring instrument: Spectrophotometer

Sensitivity: 0.78 U/g tissue

Detection range: 0.78-156 U/g tissue

Average intra-assay CV (%): 3.1

Average inter-assay CV (%): 4.6

Verage recovery rate (%): 99

- This kit is for research use only.
- Instructions should be followed strictly, changes of operation may result in unreliable results.
- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to detect Phenylalanine ammonia lyase (PAL) activity in plant tissue samples.

▲Background

Phenylalanine ammonia lyase (PAL) (EC4.3.1.5) is a key enzyme in the metabolism of phenylpropanes in plants. It is closely related to the synthesis of some important secondary substances such as lignin, isoflavones and flavonoid, and plays an important role in the normal growth and development of plants and the process of resisting the invasion of bacteria.

▲Detection principle

Phenylalanine ammonia lyase (PAL) can catalyze L-phenylalanine to produce trans-cinnamic acid and ammonia, and trans-cinnamic acid has the maximum absorption peak at 290 nm. PAL activity can be calculated by measuring the increase of OD value at 290 nm.

▲Kit components & Storage

Item	Component	Specification	Storage
Reagent 1	Extracting Solution	60 mL × 1 vial	2-8℃ , 12 months
Reagent 2	Buffer Solution	50 mL × 2 vials	2-8℃ , 12 months
Reagent 3	Substrate	Powder × 4 vials	2-8℃ , 12 months
Reagent 4	Stop Solution	6 mL × 1vial	2-8℃ , 12 months
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Spectrophotometer (290 nm), Tubes, Micropipettor, Vortex mixer, 37°C Incubator

Reagents

Double distilled water

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

After tissue homogenate and centrifugation, the supernatant must be clarified without impurities, otherwise, the supernatant must be centrifuged again until the supernatant is clarified without impurities.

Pre-assay preparation

▲Reagent preparation

Preparation of reagent 3 working solution:

Dissolve a vial of reagent 3 with 6 mL double distilled water fully and store at 2-8℃ for a month.

▲Sample preparation

10% tissue homogenate sample:

Accurately weigh the tissue sample, add reagent 1 according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min at 4°C, take the supernatant and preserve the sample on ice for detection. If there is any floating matter, take the supernatant and centrifuge it again until the supernatant is completely clarified.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.78-156 U/g tissue).

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

Sample type	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

Note: The diluent is reagent 1.

▲Detailed operating steps

The measurement of samples

1) Control tube: Take 800 μL of reagent 2, 200 μL of reagent 3 working solution into 1.5 mL EP tubes

   Sample tube: Take 20 μL of sample, 780 μL of reagent 2, 200 μL of reagent 3 working solution into 1.5 mL EP tubes.

2) Mix fully with the vortex mixer for 3 s, incubate accurately at 37℃ for 30 min.

3) Add 40 μL of reagent 4 into each tubes.

4) Mix fully with the vortex mixer for 3 s, stand for 5 min and set to zero with double distilled water and measure the OD value of each tube with 1 cm optical path cuvette at 290 nm.

▲Summary operation table

	Sample tube	Control tube
Sample (μL)	20	
Reagent 2 (μL)	780	800
Reagent 3 working solution (μL)	200	200
Mix fully, incubate accurately at 37℃ for 30 min.		
Reagent 4 (μL)	40	40
Mix fully, stand for 5 min and set to zero with double distilled water and measure the OD value at 290 nm.		

▲Calculation

Definition: 0.1 OD value changed per minute by 1 g of tissue in 1 mL of the reaction system at 37℃ that is defined as an enzyme activity unit.

PAL activity (U/g tissue)= $\Delta A_{290} \times V_2 \div 0.1^* \div t \div (m \div V_3 \times V_1)$

Note:

ΔA_{290}: OD_{sample}-$OD_{control}$;

m: weight of sample, It is recommended to take 0.05 g;

V_1: the volume of sample added to the reaction, 0.02 mL;

V_2: the total volume of the reaction system, 1.04 mL;

V_3: the volume of added reagent 1, if m=0.05, then V_3=0.45 mL;

t: enzymatic Reaction time, 30 min;

*: the absorbance value decreased by 0.1.

Appendix I Data

▲Example analysis

For green pepper, take fresh supernatant of 10% green pepper tissue homogenate, and carry the assay according to the operation table. The results are as follows:

the average OD value of the sample is 0.116, the average OD value of the control is 0.057, and the calculation result is:

PAL activity (U/g tissue)= (0.116 - 0.057) × 1.04 ÷ 0.1 ÷ 30 ÷ 0.05 × 0.45 ÷ 0.02 =9.20 U/g tissue