（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K522-S

Specification：50 Assays(48 samples)/100 Assays(98 samples)

Measuring instrument：Spectrophotometer（290 nm）

Detection range：0.78-156 U/g tissue

Elabscience® Phenylalanine Ammonia Lyase (PAL) Activity Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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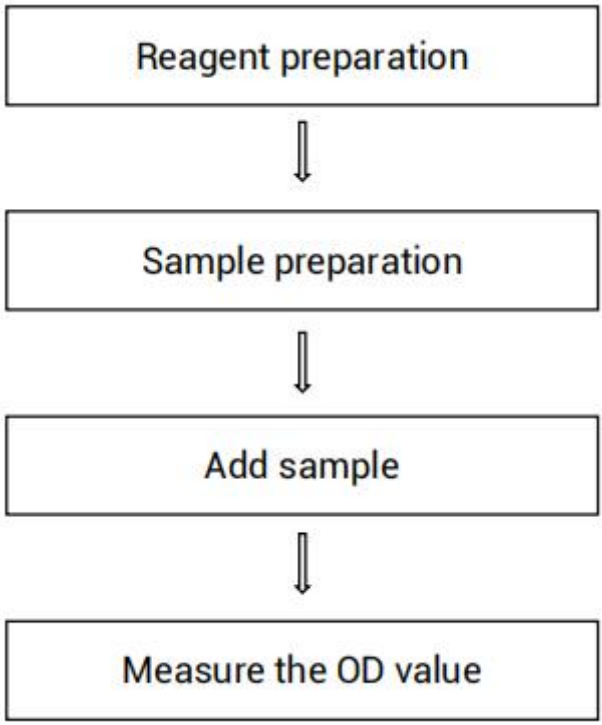
Assay summary



Intended use

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

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	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Extracting Solution	30 mL × 1 vial	60 mL × 1 vial	2-8°C, 12 months
Reagent 2	Buffer Solution	50 mL × 1 vial	50 mL × 2 vials	2-8°C, 12 months
Reagent 3	Substrate	Powder × 2 vials	Powder × 4 vials	2-8°C, 12 months
Reagent 4	Stop Solution	3 mL × 1vial	6 mL × 1vial	2-8°C, 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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

Double distilled water

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of substrate working solution：
Dissolve a vial of substrate with 6 mL double distilled water. Store at 2~8°C for 1 month.

Sample preparation

① Sample preparation

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL extracting solution with a dounce homogenizer at 4°C .

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ If there is any floating matter, take the supernatant and centrifuge it again until the supernatant is completely clarified.

② **Dilution of sample**

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 extracting solution. For the dilution of other sample types, please do pretest to confirm the dilution factor

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.

Operating steps

① Control tube: Take 800 μL of buffer solution, 200 μL of substrate working solution into 1.5 mL EP tubes
Sample tube: Take 20 μL of sample, 780 μL of buffer solution, 200 μL of substrate working solution into 1.5 mL EP tubes.

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

③ Add 40 μL of stop solution 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.

Calculation

The sample:

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

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

[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 extracting solution, if m=0.05, then V_3=0.45 mL;

t: enzymatic Reaction time, 30 min;

*: the absorbance value decreased by 0.1.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/g tissue)	2.80	54.20	123.40
%CV	3.8	3.1	2.4

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/g tissue)	2.80	54.20	123.40
%CV	4.1	4.3	5.4

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 99%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/g tissue)	12	68.5	108
Observed Conc.(U/g tissue)	11.9	69.2	104.8
Recovery rate (%)	99	101	97

Sensitivity

The analytical sensitivity of the assay is 0.78 U/g tissue. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix Π Example Analysis

Example analysis：

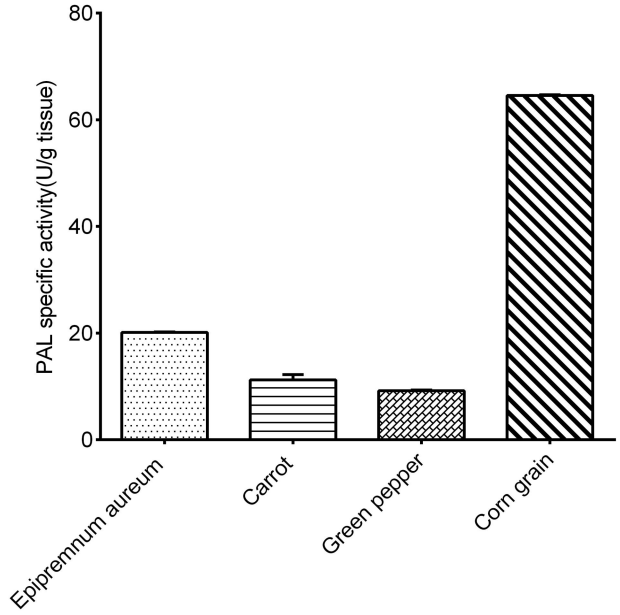
For green pepper, take fresh supernatant of 10% green pepper tissue homogenate, and carry the assay according to the operation steps. The results are as follows:

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

Detect 10% epipremnum aureum tissue homogenate, 10% carrot tissue homogenate, 10% green pepper tissue homogenate and 10% corn grain tissue homogenate according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.