TECHNICAL MANUAL

Phenylalanine Ammonia Lyase (PAL) Activity Assay Kit

- **SKU CODE:** MAES0426
- **SIZE:** 100 Assays
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add reagents to tubes
- Incubate at 37°C
- Add stop solution
- Measure absorbance at 290 nm

2. Intended use

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

3. Detection principle

Phenylalanine ammonia lyase (PAL) catalyzes L-phenylalanine to produce trans-cinnamic acid and ammonia. Trans-cinnamic acid has a maximum absorption peak at 290 nm. PAL activity can be calculated by measuring the increase in optical density at 290 nm.

4. 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 × 1 vial	6 mL × 1 vial	2-8°C, 12 months

5. Materials prepared by users

Instruments:

Spectrophotometer (290 nm), tubes, micropipette, vortex mixer, 37°C incubator

Reagents:

Double distilled water

6. Reagent preparation

1. Allow all reagents to equilibrate to room temperature before use.
2. **Preparation of substrate working solution:** Dissolve one vial of substrate with 6 mL double distilled water. Store at 2-8°C for 1 month.

7. 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 10,000×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. **Sample dilution:** The recommended dilution factor for different samples is as follows (for reference only): - 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 dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

After tissue homogenization and centrifugation, the supernatant must be clarified without impurities. Otherwise, the supernatant must be centrifuged again until it is clarified without impurities.

9. Operating steps

1. Control tube: Add 800 µL of buffer solution and 200 µL of substrate working solution into 1.5 mL EP tubes. Sample tube: Add 20 µL of sample, 780 µL of buffer solution, and 200 µL of substrate working solution into 1.5 mL EP tubes.
2. Mix thoroughly with the vortex mixer for 3 seconds, then incubate at 37°C for 30 minutes.

3. Add 40 µL of stop solution to each tube.
4. Mix thoroughly with the vortex mixer for 3 seconds, stand for 5 minutes, then set to zero with double distilled water and measure the OD value of each tube with a 1 cm optical path cuvette at 290 nm.

10. Calculation

Definition: One unit of enzyme activity is defined as the amount of enzyme that causes a 0.1 OD change per minute per gram of tissue in 1 mL of reaction system at 37°C.

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 (recommended 0.05 g)

V_1: volume of sample added to the reaction (0.02 mL)

V_2: total volume of the reaction system (1.04 mL)

V_3: volume of extracting solution added (if m=0.05 g, 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

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision

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.

Inter-assay Precision

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

Three samples of high, medium, and low concentrations were tested six times in parallel for each concentration to obtain an average recovery rate of 99%.

Recovery

	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.

12. Appendix II Example Analysis

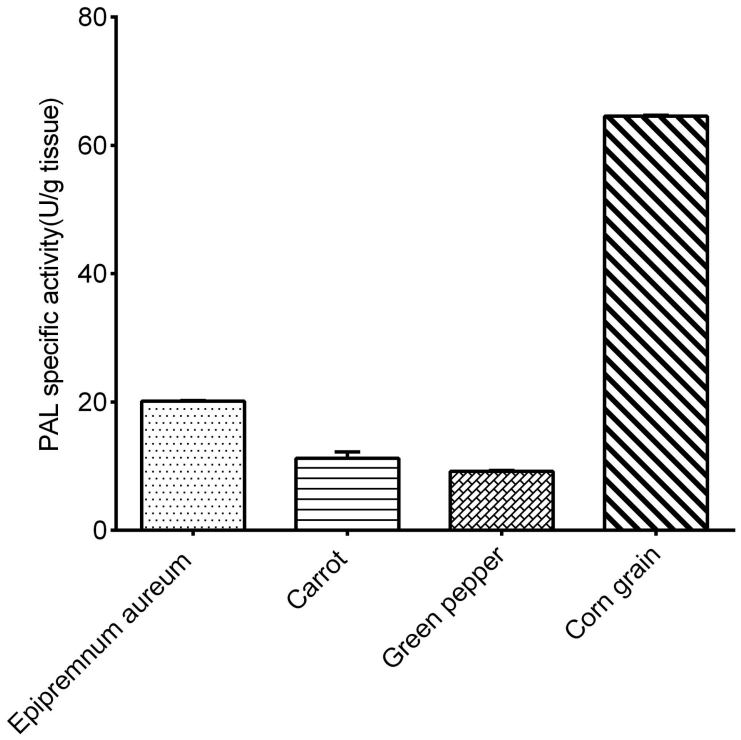
Example analysis:

For green pepper, fresh supernatant of 10% green pepper tissue homogenate was used, and the assay was carried out according to the operating 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

Detection of 10% epipremnum aureum tissue homogenate, 10% carrot tissue homogenate, 10% green pepper tissue homogenate, and 10% corn grain tissue homogenate according to the protocol showed the following results:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.