CheKine™ Micro Phenylalanine Ammonia Lyase (PAL) Activity Assay Kit

Cat #: KTB1160 Size: 48 T/96 T

	Micro Phenylalanine Ammonia Lyase (PAL) Activity Assay Kit		
REF	**Cat #**: KTB1160	LOT	**Lot #:** Refer to product label
	Applicable samples: Plant Tissues		
	Storage: Stored for 6 months at 4℃		

Assay Principle

Phenylalanine Ammonia Lyase (PAL, EC4. 3 15) is widely present in various plants and a few microorganisms. It is a key enzyme and rate-limiting enzyme of phenylpropane metabolism in plants, and has not been found in animals. PAL is closely related to the synthesis of some important secondary substances such as lignin, isoflavone phytoales, and flavonoid pigments, and plays an important role in the normal growth and development of plants, disease resistance, and stress resistance. PAL catalyzes the cleavage of L-phenylalanine into trans-cinnamic acid and ammonia. Trans-cinnamic acid has a maximum absorption value at 290 nm. PAL activity is calculated by measuring the rate of increase in absorbance.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Assay Buffer	7.5 mL	15 mL	4℃
Substrate	1	1	4℃
HCl	0.5 mL	1 mL	4℃

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 290 nm
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Incubator, refrigerated centrifuge
- Deionized water
- Homogenizer

Reagent Preparation

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Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Working Substrate: Add deionized water into substrate, and fully dissolve it for later use. (48 T: add 2 mL deionized water into substrate; 96 T: add 4 mL deionized water into substrate). The remaining solution can be stored at 4℃ for one week.

HCl: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Sample Preparation

According to the tissue weight (g): Weigh about 0.1 g tissue and add 1 mL Extraction Buffer. Homogenize on ice. Centrifuge at 10,000 g for 10 min at 4℃, aspirating the supernatant, place it on ice to be tested.

Note: It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 290 nm, ultraviolet spectrophotometer was returned to zero with deionized water.

2. Sample measurement (add the following reagents in sequence into the 96-well UV plate or microquartz cuvette).

Reagent	Test Well (μL)	Blank Well (μL)
Sample	5	0
Assay Buffer	145	150
Working Substrate	40	40
Mix well and react at 30°C for 30 min		
HCl	10	10

3. Mix well and keep at room temperature for 10 min. Record the absorbance value of test well as A_1 and blank well as A_2 at 290 nm. $\Delta A=A_1-A_2$.

Note: Only one blank well needs to be made. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.001, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.5, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well UV plates calculation formula

1. Calculated by fresh weight of samples

Unit definition: One unit defines as the change of absorbance at 290 nm by 0.05 per h per g of tissue sample.

PAL (U/g fresh weight)=$\Delta A\times V_{Total}\div(V_{Sample}\div V_{Sample\ Total}\times W)\div0.05\div T$**=26.67×ΔA÷W**

2. Calculated by protein concentration

Unit definition: One unit defines as the change of absorbance at 290 nm by 0.05 per min per mg of tissue sample.

PAL (U/mg prot)=$\Delta A\times V_{Total}\div(Cpr\times V_{Sample})\div0.05\div T$**=26.67×ΔA÷Cpr**

B. microquartz cuvette calculation formula

1. Calculated by fresh weight of samples

Unit definition: One unit defines as the change of absorbance at 290 nm by 0.1 per h per g of tissue sample.

PAL (U/g fresh weight)=$\Delta A\times V_{Total}\div(V_{Sample}\div V_{Sample\ Total}\times W)\div0.1\div T$**=13.33×ΔA÷W**

2. Calculated by protein concentration

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Unit definition: One unit defines as the change of absorbance at 290 nm by 0.1 per min per mg of tissue sample.

PAL (U/mg prot)=$\Delta A \times V_{Total} \div (Cpr \times V_{Sample}) \div 0.1 \div T$**=13.33×ΔA÷Cpr**

Where: V_{Total}: Total reaction volume, 200 μL; V_{Sample}: Sample volume added, 5 μL; $V_{Sample\ Total}$: Extract Buffer added to samples, 1 mL; W: sample weight, g; T: Reaction time, 30 min; Cpr: Sample protein concentration, mg/mL.

Recommended Products

Catalog No.	Product Name
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Activity Assay Kit
KTB1040	CheKine™ Micro Catalase (CAT) Activity Assay Kit
KTB1140	CheKine™ Micro Polyphenol Oxidase (PPO) Activity Assay Kit

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The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.

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