

## Phenylalanine Ammonia-lyase (PAL) Activity Assay Kit

**Note:** It is necessary to predict 2-3 large difference samples before the formal determination.

**Operation Equipment:** Spectrophotometer

**Catalog Number:** BC0210

**Size:** 50T/48S

**Product Composition:** Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 60 mL×1	2-8°C
Reagent I	Liquid 40 mL×1	2-8°C
Reagent II	Powder×3	2-8°C
Reagent III	Liquid 5 mL×1	2-8°C

### Solution Preparation:

1. Reagent II: Dissolve with 4 mL of distilled water one of the bottle before using, and unused liquid can be stored at 2-8°C for 2 weeks.

### Product Description:

Phenylalanine Ammonia-lyase (PAL) is widely found in various plants and a few microorganisms. It is a key enzyme in phenylpropanoid metabolism. PAL is closely related to some important secondary substances synthetic such as lignin, isoflavones phytoalexin, flavonoid pigments, and play an important role in normal growth and development in plants and against the bacteria resist.

L-phenylalanine can be decomposed into trans-cinnamic acid and ammonia by PAL, and trans-cinnamic acid has the maximum absorption value at 290 nm. In this kit, the activity of PAL can be calculated by measuring the absorbance increased rate.

### Reagents and Equipments Required but Not Provided:

Ultraviolet spectrophotometer, water bath, refrigerated centrifuge, transferpettor, 1 mL quartz cuvette, mortar /homogenizer, ice and distilled water.

### Procedure:

#### I. Sample preparation:

Add 1 mL of Extract solution into 0.1 g of tissue, and fully homogenized on ice. Centrifuge at 10000 ×g for 10 minutes at 4°C to remove insoluble materials and take the supernatant on ice for testing.

#### II. Determination procedure:

- Preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 290 nm, and set the counter to zero with distilled water.
- Add the reagents as following

Reagent (μL)	Test tube (A1)	Contrast tube (A2)
Sample	20	-

Reagent I	780	800
Reagent II	200	200
Mix thoroughly, incubate at 30°C for 30 minutes.		
Reagent III	40	40

Mix thoroughly and place for 10 minutes. Detect the absorbance of the test tube (A1) and the contrast tube (A2) at 290 nm, calculate  $\Delta A = A1 - A2$ .

**Note:** Contrast tube just needs to test once or twice.

### III. Calculation:

#### 1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance of 0.1 change at 290 nm in per milliliter reaction system per minute every milligram protein.

$$\text{PAL(U/mg prot)} = \Delta A \times V_{rv} \div V_s \div T \div 0.1 \div \text{Cpr} = 17.3 \times \Delta A \div \text{Cpr}$$

#### 2. Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance of 0.1 change at 290 nm in per milliliter reaction system per minute every gram tissue.

$$\text{PAL(U/g weight)} = \Delta A \times V_{rv} \div (W \times V_s \div V) \div T \div 0.1 = 17.3 \times \Delta A \div W$$

Cpr: Sample concentration, mg/mL;

W: Sample weight, g;

Vs: Sample volume, 20  $\mu\text{L}$  = 0.02 mL;

V: Total sample volume, 1 mL

Vrv: Total reaction volume, 1040  $\mu\text{L}$  = 1.04 mL;

T: Reaction time, 30 minutes.

### Recent Product Citations:

[1] Zheng XR, Zhang MJ, Qiao YH, Li R, Alkan N, Chen JY, Chen FM. Cyclocarya paliurus Reprograms the Flavonoid Biosynthesis Pathway Against Colletotrichum fructicola. *Front Plant Sci.* 2022 Jun 30;13:933484. doi: 10.3389/fpls.2022.933484. PMID: 35845688; PMCID: PMC9280340.

[2] Li Y, Xu L, Ma H, Su Y, Zhang Q, Zhao Y, Wang M. Design, Synthesis, and Fungicidal Activity of Novel Plant Elicitors Based on a Diversity-Oriented Synthesis Strategy. *J Agric Food Chem.* 2022 Oct 26;70(42):13486-13498. doi: 10.1021/acs.jafc.2c04013. Epub 2022 Oct 18. PMID: 36254833.

[3] Jia L, Li Y, Liu G, He J. UV-C delays senescence in 'Lingwu long' jujube fruit by regulating ROS and phenylpropanoid metabolism. *Plant Physiol Biochem.* 2023 Jan;194:383-393. doi: 10.1016/j.plaphy.2022.11.030. Epub 2022 Nov 26. PMID: 36473328.

[4] Sun S, Chen L, Huo J, Wang Y, Kou S, Yuan S, Fu Y, Zhang J. Discovery of Novel Pyrazole Amides as Potent Fungicide Candidates and Evaluation of Their Mode of Action. *J Agric Food Chem.*

2022 Mar 23;70(11):3447-3457. doi: 10.1021/acs.jafc.2c00092. Epub 2022 Mar 13. PMID: 35282681.

### References:

- [1] Aydaş S B, Ozturk S, Aslım B. Phenylalanine ammonia lyase (PAL) enzyme activity and antioxidant properties of some cyanobacteria isolates[J]. Food chemistry, 2013, 136(1): 164-169.
- [2] Rosler J, Krekel F, Amrhein N, et al. Maize phenylalanine ammonia-lyase has tyrosine ammonia-lyase activity[J]. Plant physiology, 1997, 113(1): 175-179.
- [3] Cheng G W, Breen P J. Activity of phenylalanine ammonia-lyase (PAL) and concentrations of anthocyanins and phenolics in developing strawberry fruit[J]. Journal of the American Society for Horticultural Science, 1991, 116(5): 865-869.

### Related Products:

- BC0190/BC0195 Polyphenol Oxidase(PPO) Activity Assay Kit
- BC0170/BC0175 Superoxide Dismutase(SOD) Activity Assay Kit
- BC0200/BC0205 Catalase(CAT) Activity Assay Kit
- BC0090/BC0095 Peroxidase(POD) Activity Assay Kit