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/ Microplate Reader

Catalog Number: BC0215

Size: 100T/96S

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 110 mL×1	2-8℃
Reagent I	Liquid 15 mL×1	2-8℃
Reagent II	Powder×2	2-8℃
Reagent III	Liquid 1 mL×1	2-8℃

Solution Preparation:

1. Reagent II: Dissolve with 2.5 mL of distilled water one of the bottle before using, and unused liquid can be stored at 2-8℃ 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 plants 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 Equipment Required but Not Provided

Ultraviolet spectrophotometer/microplate reader, refrigerated centrifuge, transferpettor, ultra-micro quartz cuvette/96 well UVflat-bottom plate, water bath/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℃ to remove insoluble materials and take the supernatant on ice for testing.

II. Determination procedure:

1. Preheat the spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to 290 nm, and set the counter to zero with distilled water.
2. Add the reagents as following to EP tube or 96 well UV flat-bottom plate

Reagent (μL)	Test tube (A1)	Contrast tube (A2)

Sample	5	-
Reagent I	145	150
Reagent II	40	40
Mix thoroughly, incubate at 30℃ for 30 minutes.		
Reagent III	10	10

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

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

III. Calculation:

1. Micro glass cuvette

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.

PAL (U/mg prot) = ΔA×Vsv÷Vs÷T÷0.1÷Cpr=13.33×ΔA ÷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.

PAL (U/g weight) = ΔA×Vsv÷Vs÷T÷0.1÷W=13.33×ΔA÷W

2. 96 well UV flat-bottom plate

1) Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance of 0.05 change at 290 nm in per milliliter reaction system per minute every milligram protein.

PAL (U/mg prot) = ΔA×Vsv÷Vs÷T÷0.05÷Cpr=26.67×ΔA ÷Cpr

2) Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance of 0.05 change at 290 nm in per milliliter reaction system per minute every gram tissue.

PAL (U/g weight) = ΔA×Vsv÷Vs÷T÷0.05÷W=26.67×ΔA÷W

Cpr: Sample concentration, mg/mL;
W: Tissue weight, g;
Vs: Sample volume, 5 μL=0.005 mL;
Vrv: Total reaction volume, 0.2 mL;
Vsv: Extraction volume, 1 mL;
T: Reaction time, 30 minutes.

Recent Product Citations:

[1] Chen Y, Li D, Zhang X, Ma Q, Xu Y, Luo Z. Azacytidine-induced hypomethylation delays

senescence and coloration in harvested strawberries by stimulating antioxidant enzymes and modulating abscisate metabolism to minimize anthocyanin overproduction. Food Chem. 2023 May 1;407:135189. doi: 10.1016/j.foodchem.2022.135189. Epub 2022 Dec 10. PMID: 36525805.

[2] Jiang N, Wang L, Jiang D, Wang M, Yu H, Yao W. Combined metabolome and transcriptome analysis reveal the mechanism of eugenol inhibition of Aspergillus carbonarius growth in table grapes (Vitis vinifera L.). Food Res Int. 2023 Aug;170:112934. doi: 10.1016/j.foodres.2023.112934. Epub 2023 May 3. PMID: 37316002.

[3] Zeng J, Chen C, Chen M, Chen J. Comparative transcriptomic and metabolomic analyses reveal the delaying effect of naringin on postharvest decay in citrus fruit. Front Plant Sci. 2022 Nov 30;13:1045857. doi: 10.3389/fpls.2022.1045857. PMID: 36531365; PMCID: PMC9748555.

[4] Zhu F, Fang Y, Wang Z, Wang P, Yang K, Xiao L, Wang R. Salicylic acid remodeling of the rhizosphere microbiome induces watermelon root resistance against Fusarium oxysporum f. sp. niveum infection. Front Microbiol. 2022 Sep 23;13:1015038. doi: 10.3389/fmicb.2022.1015038. PMID: 36212858; PMCID: PMC9539938.

[5] Xu W, Yang Q, Yang F, Xie X, Goodwin PH, Deng X, Tian B, Yang L. Evaluation and Genome Analysis of Bacillus subtilis YB-04 as a Potential Biocontrol Agent Against Fusarium Wilt and Growth Promotion Agent of Cucumber. Front Microbiol. 2022 Jun 9;13:885430. doi: 10.3389/fmicb.2022.885430. PMID: 35756052; PMCID: PMC9218633.

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