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# 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:** AK0577

**Size:** 100T/96S

**Components:**

**Extract solution:** Liquid 110 mL×1. Storage at 4℃ .

**Reagent I:** Liquid 15 mL×1. Storage at 4℃ .

**Reagent II:** Powder×2. Storage at 4℃ . Dissolve with 1.5 mL of distilled water before use. The reagent should be prepared just before use, and could be stored at 4℃ for 2 weeks.

**Reagent III:** Liquid 1 mL×1. Storage at 4℃ .

**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, 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] Li B, Ding Y, Tang X, et al. Effect of L-Arginine on Maintaining Storage Quality of the White Button Mushroom (Agaricus bisporus) [J]. Food and Bioprocess Technology, 2019, 12(4): 563-574.

[2] Zhang J, Lv J, Xie J, et al. Nitrogen Source Affects the Composition of Metabolites in Pepper (Capsicum annuum L.) and Regulates the Synthesis of Capsaicinoids through the GOGAT–GS Pathway[J]. Foods, 2020, 9(2): 150.

[2] Zhang J, Lv J, Xie J, et al. Nitrogen Source Affects the Composition of Metabolites in Pepper (Capsicum annuum L.) and Regulates the Synthesis of Capsaicinoids through the GOGAT–GS Pathway[J]. Foods, 2020, 9(2): 150.

**References:**

[1] Aydaş S B, Ozturk S, Asl1m 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:**

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|---|---|
| AK0293/AK0292 | Polyphenol Oxidase(PPO) Activity Assay Kit |
| AK0584/AK0583 | Superoxide Dismutase(SOD) Activity Assay Kit |
| AK0580/AK0579 | Catalase(CAT) Activity Assay Kit |
| AK0598/AK0597 | Peroxidase(POD) Activity Assay Kit |