Phenylalanine ammonia-lyase Microplate Assay Kit User Manual

Catalog # ASK1018

Detection and Quantification of Phenylalanine ammonia-lyase (PAL) activity in Tissue extracts, Cell lysate Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

PAL widely found in various plants and a few micro-organisms, is a key enzyme in plants phenylpropanoid metabolism, and 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.

PAL catalytic cracking L- phenylalanine for trans-cinnamic acid and ammonia, trans-cinnamic acid has the maximum absorption value at 290 nm, PAL activity is calculated by measuring the absorbance increased rate.

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II. KIT COMPONENTS

Component	Volume	Storage
96-Well UV Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	30 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Stop Solution	4 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate: add 10 ml Distilled water to dissolve before use, store at 4 °C.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 290 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

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IV. SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

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V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Control
Sample	10 µl	--
Reaction Buffer	120 µl	120 µl
Substrate	50 µl	50 µl
Mix, put it in the oven, 30 °C for 30 minutes.		
Stop Solution	20 µl	20 µl
Sample	--	10 µl
Mix, record absorbance measured at 290nm immediately.		

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VI. CALCULATION

Unit Definition: one unit is defined as the OD value changed 0.01 in the reaction system per minute.

1. According to the protein concentration of sample

$$PAL\ (U/mg) = (OD_{Sample} - OD_{Control}) \times V_{Total} / (V_{Sample} \times C_{Protein}) / 0.01 / T$$

$$= 66.7 \times (OD_{Sample} - OD_{Control}) / C_{Protein}$$

2. According to the weight of sample

$$PAL\ (U/g) = (OD_{Sample} - OD_{Control}) \times V_{Total} / (V_{Sample} \times W / V_{Assay}) / 0.01 / T$$

$$= 66.7 \times (OD_{Sample} - OD_{Control}) / W$$

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

V_{Total}: the total volume of the enzymatic reaction, 0.2 ml;

V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 30 minutes.

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