Phenylalanine ammonia-lyase

Microplate Assay Kit

User Manual

Catalog # CAK1018

(Version 1.1E)

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.

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.

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, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

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.

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.		

1) Reagents must be added step by step, can not be mixed and added together.

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.

VII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

VIII. NOTES