Phenylalanine ammonia-lyase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1018

(Version 2.2F)

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.

Phenylalanine ammonia-lyase Activity Colorimetric Microplate Assay Kit provides a convenient tool for sensitive detection of phenylalanine ammonia-lyase activity in a variety of samples. PAL catalytic will decompose L-phenylalanine to trans-cinnamic acid and ammonia. The ammonia reacts with the dyeto form a colored product.The color intensity increaseat 620nm is proportional to PALactivity in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	10 ml x 1	4 °C
Substrate	Powder x 1	4 °C, keep in dark
Positive Control	Powder x 1	-20 °C
Standard	Powder x 1	4 °C
Dye Reagent A	Powder x 2	4 °C, keep in dark
Dye Reagent B	Powder x 2	4 °C, keep in dark
Dye Reagent B Diluent	3 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 620 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. 1.5 ml microcentrifuge tube
8. Centrifuge
9. Timer

IV. REAGENT PREPARATION

Positive Control: Briefly centrifuge prior to opening. Dissolvein50µLAssay Bufferbefore use.Store at -20 °C for 1 month.

Substrate: Briefly centrifuge prior to opening. Dissolvein1 mlReaction Buffer before use, store at 4 °C.

Dye Reagent A: Briefly centrifuge prior to opening.Add 3.5 ml distilled waterinto 1 vialDye Reagent A to dissolve before use. Store at 4 °Cin dark and use within 24 hours.

Dye Reagent B: Briefly centrifuge prior to opening. Add 1.5 ml Dye Reagent B Diluentinto 1 vial Dye Reagent B,mix before use. Store at 4 °Cin dark and use within 24 hours.

Standard: Briefly centrifuge prior to opening. Add 1 ml distilled water to dissolve, then add 5 µL into 995 µLdistilled water, mix, the concentration will be 1000 µmol/L.Store at 4°C for 1 month.Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 1000/500/250/125/62.5/31.2/15.6 µmol/L.

V. SAMPLE PREPARATION

1.For tissue samples

Weighout 0.1 g tissue, homogenize with 1mlAssay Buffer on ice, centrifuged at 8000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

VI. ASSAY PROCEDURE

Add following reagents into1.5 ml microcentrifuge tube first

Reagent*	Sample**	Control	Positive Control	Standard	Blank
Reaction Buffer	80 µl	80 µl	80 µl	--	--
Distilled water	--	10 µl	--	--	100 µl
Sample	10 µl	--	--	--	--
Positive control	--	--	10 µl	--	--
Substrate	10 µl	10 µl	10 µl	--	--
Standard	--	--	--	100 µl	--
Shake and mix, put it into the oven,37°C for 45minutes					
Dye ReagentA	70 µl	70 µl	70 µl	70 µl	70 µl
Dye ReagentB	30 µl	30 µl	30 µl	30 µl	30 µl
Shake and mix, centrifuged at 10,000 rpm for 5minutes, transfer150 µlsupernatant into the microplate for detection.Record absorbance measured at 620 nm.					

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit is defined as the amount of the enzyme willcatalyze the deaminationof L-phenylalanine to produce1μmoleammonia per minute.

1. Calculatethe sample activity in ASSAY PROCEDURE according to the slope of the standard curve

$$\text{Activity}=\frac{(OD_{Sample}-OD_{Control})-\text{Intercept}}{\text{Slope}\times T}\times n(U/mL)$$

Calculate the initial activity according to sample preparation procedure.

2. According to one point of the standard OD value and concentration

2.1According to the protein concentration of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Control})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}\times C_{Protein}\times T}(U/mg)$$

2.2According to the weight of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Control})}{(OD_{Standard}-OD_{Blank})\times(W\times V_{Sample}/V_{Assay})\times T}(U/g)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

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

$C_{Standard}$: the concentration of standard, μmol/mL

W: the weight of total sample, g

$V_{Standard}$: the volume of standard in assay procedure, mL

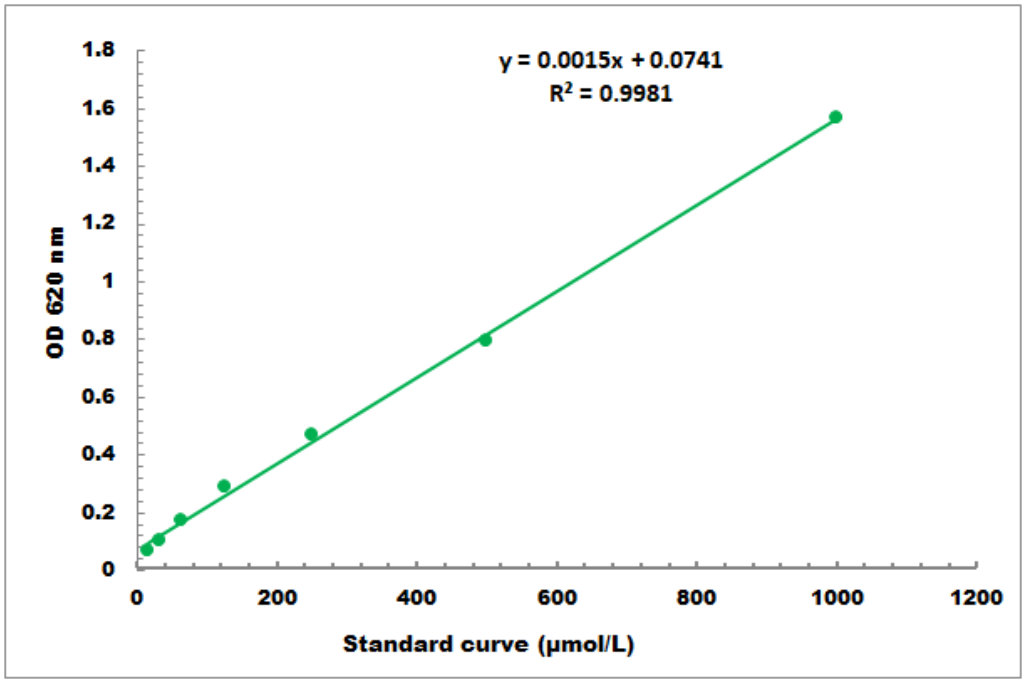
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

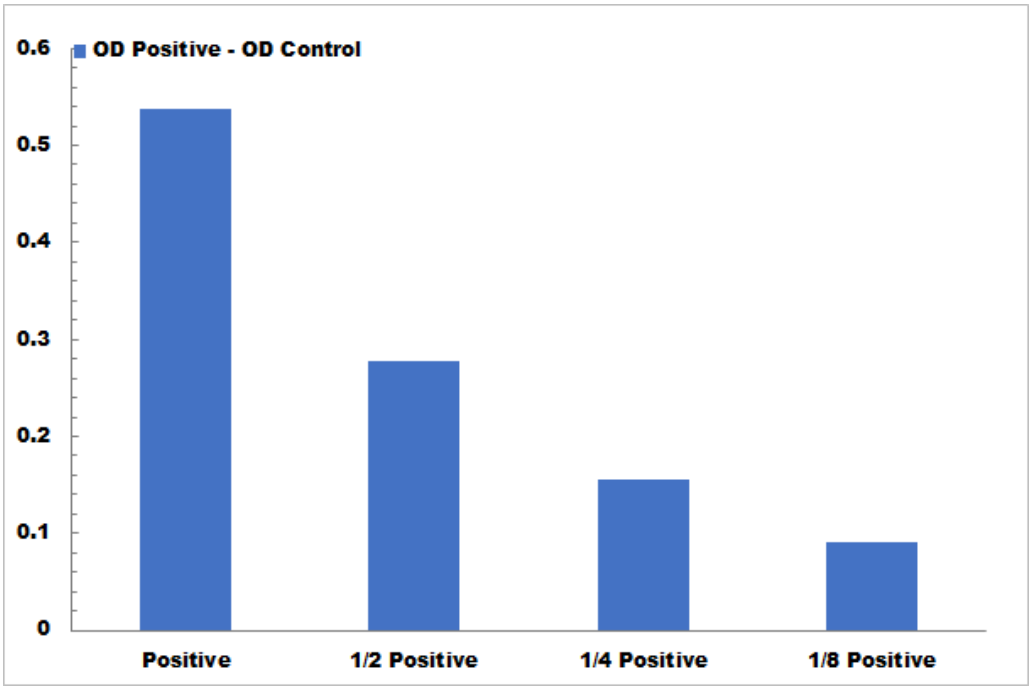
T: the reaction time in step 1, minute

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 10μmol/L - 1000μmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration