Phenylalanine ammonia-lyase Activity Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6627

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 .

1. 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 dye to form a colored product. The color intensity increase at 620 nm is proportional to PAL activity in the sample.

2. 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	-

3. 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

4. Reagent Preparation

Positive Control: Briefly centrifuge prior to opening. Dissolve in 50 µL Assay Buffer before use. Store at -20°C for 1 month.

Substrate: Briefly centrifuge prior to opening. Dissolve in 1 mL Reaction Buffer before use, store at 4°C.

Dye Reagent A: Briefly centrifuge prior to opening. Add 3.5 mL distilled water into 1 vial Dye Reagent A to dissolve before use. Store at 4°C in dark and use within 24 hours.

Dye Reagent B: Briefly centrifuge prior to opening. Add 1.5 mL Dye Reagent B Diluent into 1 vial Dye Reagent B , mix before use. Store at 4°C in 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 µL distilled water , mix, the concentration will be 1000 µmol/L. Store at 4°C for 1 month. Perform 2 -fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 1000/500/250/125/62. 5/31.2/15.6 µmol/ L.

5. Sample Preparation

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

6. Assay Procedure

Add following reagents in to 1.5 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 45 minutes					
Dye Reagent A	70 µl	70 µl	70 µl	70 µl	70 µl
Dye Reagent B	30 µl	30 µl	30 µl	30 µl	30 µl
Shake and mix, centrifuged at 10,000 rpm for 5 minutes, transfer 150 µl supernatant 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.

7. Calculation

Unit Definition:

One unit is defined as the amount of the enzyme will catalyze the

deamination of L-phenylalanine to produce 1 μmol ammonia per minute .

1. Calculate the sample activity in ASSAY PROCEDURE according to the slope of the

standard curve

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

Calculate the initial activity according to sample preparation procedure .

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

2.1 According to the protein concentration of sample

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

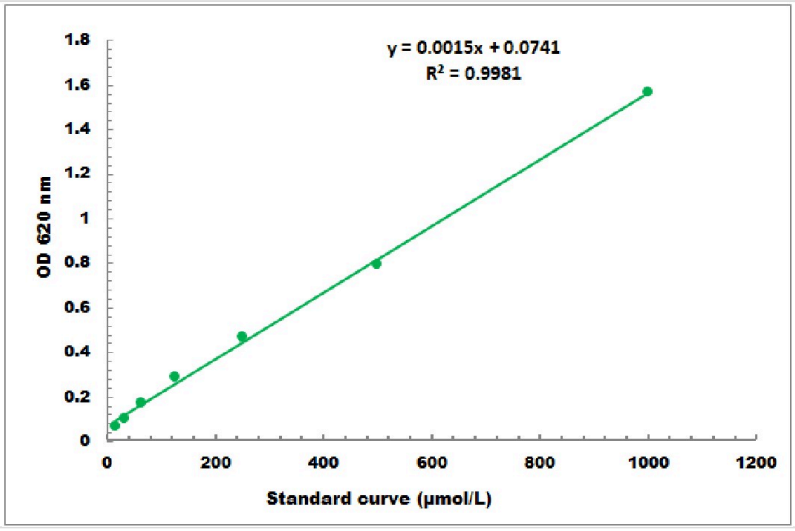
2.2 According to the weight of sample

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

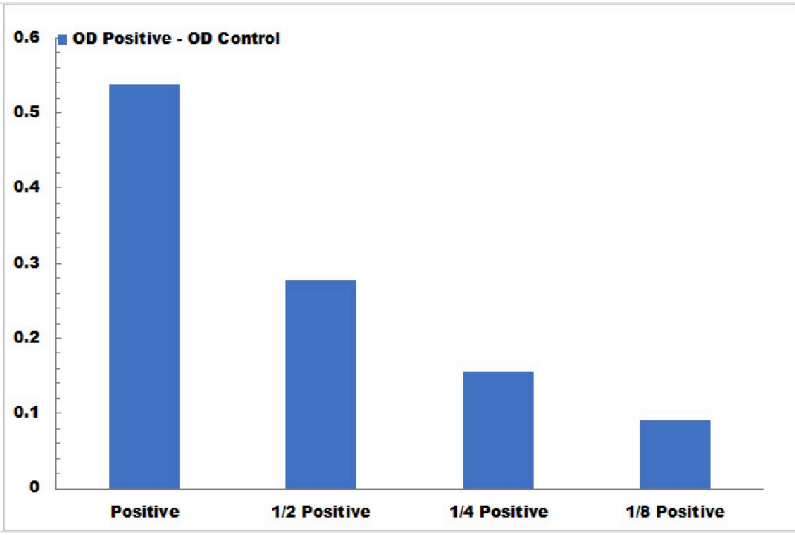
Item	Definition
Slope	the absorbance slope of standard curve
n	the dilution factor
$C_{Protein}$	the protein concentration, mg/mL
$C_{Standard}$	the concentration of standard, μmol/mL
W	the weight of total sample , g
$V_{Standard}$	the volume of standard in assay procedure, mL
V_{Sample}	the volume of sample in assay procedure, mL
V_{Assay}	the volume of Assay Buffer in sample preparation , mL
T	the reaction time in step 1 , minute

8. Typical Data

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



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



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