Phytase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1152

(Version 1.4A)

Detection and Quantification of PhytaseActivity in Tissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

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

I. INTRODUCTION

Phytase is a phosphatase enzyme that catalyzes, or kickstarts, the hydrolysis of phytic acid, also known as phytate. Phytic acid is a form of indigestible phosphorus that's present in plant-based foods such as cereal, wheat, and various grains. Roughly two-thirds of the phosphorus present in plant foods is bound in the form of phytic phosphate. Through the hydrolysis reaction, phytase liberates phosphorus so that the body can use it. While phytases have been found to occur in animals, plants, fungi and bacteria, phytases have been most commonly detected and characterized from fungi.

Phytase Activity Colorimetric Microplate Assay Kit is designed to measure pytase activity in biological samples. Phytase decomposes the substrate. The dye reagent forms a color with released phosphate ion, which is measured on a plate reader 660 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-WellMicroplate	1 plate	
Assay Buffer	30mlx 4	4 °C
Reaction Buffer	8 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Dye Reagent I	Powder x 1	4 °C
Dye Reagent II	Powder x 1	4 °C
Dye Reagent III	10 ml x 1	4 °C
Standard (1mmol/L)	1 ml x 1	-20 °C
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 660 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice
9. Convection oven

IV. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 1000/500/250/125/62.5/31.2/15.6μmol/L.

Substrate: Add 8 ml Reaction Buffer into Substrate, mix.Store at 4 °C for 1-2 months or -20°C for 3-6months after reconstitution.

Dye Reagent: Add 5 ml Dye Reagent III into Dye Reagent I and 1 ml Dye Reagent III into Dye Reagent II respectivelyto dissolve. Transfer allDye Reagent IIinto Dye Reagent III, mix; then transfer all Dye Reagent I into Dye Reagent III (Must follow this step).The mixed Dye Reagent may store at 4 °C for 2-3 days.

***Note:**It should be yellow. If colorless, the solution is failure. If blue, the solutionispolluted.This solution should be preparedbefore use.It is best to usedisposable plastic containers to prepare the solution in order to prevent phosphoruspollution.

Positive Control: Briefly centrifuge prior to opening. Add 1 ml distilled waterto dissolve before use, then add 0.1 ml into 0.9 ml distilled water.Store at -80°C for 1 month after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay Buffer for 5 ×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 8000g 4°C for 20minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay Buffer on ice, centrifuged at 8000g 4°C for 20minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

VI. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent*	Sample**	Control	Standard	Blank	Positive Control
Substrate	80 µl	80 µl	--	--	80 µl
Sample	20 µl	--	--	--	--
Distilled water	--	20 µl	--	100 µl	--
Positive Control	--	--	--	--	20 µl
Standard	--	--	100 µl	--	--
Mix, cover the plate adhesive strip, put the plate into the convection oven, incubate at 55 °C for 10 minutes.					
Dye Reagent	100µl	100 µl	100 µl	100 µl	100 µl
Mix, measured at 660 nm and record the absorbance.					

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 of phytaseactivity is defined as the enzymethe enzyme generates 1μmol of PO_4^{3-} per minute.

1. According to the slope of the standard curve

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

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

2.1 According 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 quantity of cells or bacteria

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times N\times(V_{Sample}/ V_{Assay})\times T}(U/10^4)$$

2.3 According 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 of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10^4

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

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

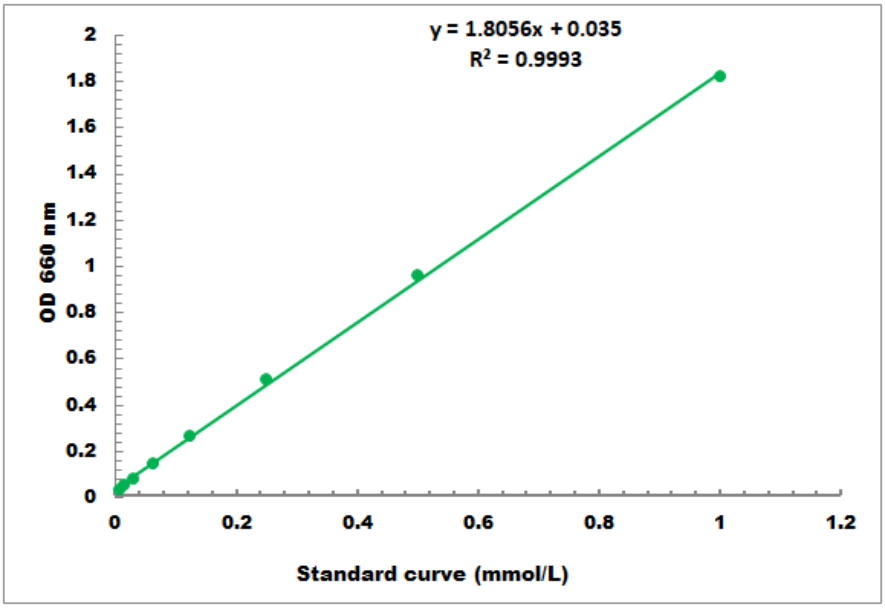
V_{Sample}: the volume of sample in assay procedure, mL

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

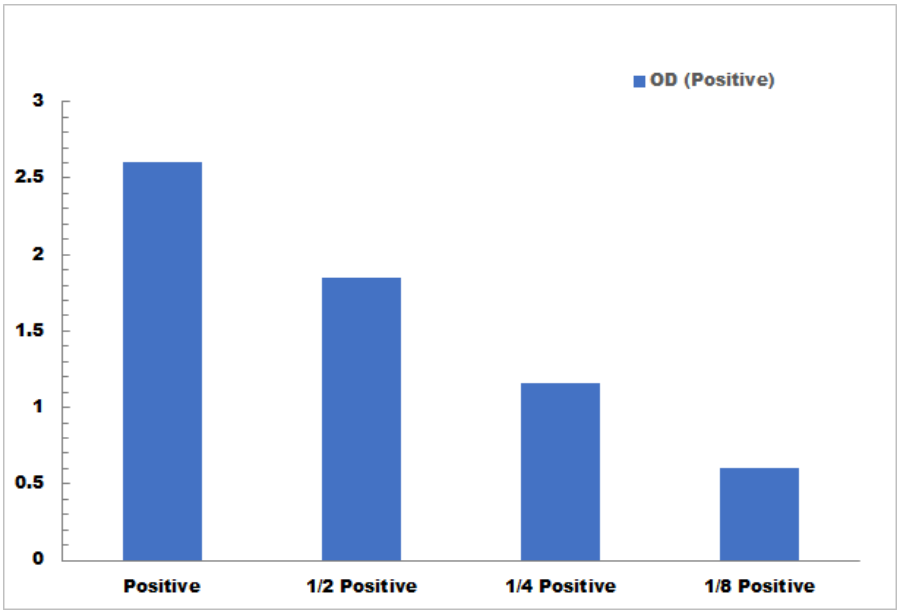
T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 0.01mmol/L -1mmol/L



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