Phytase Microplate Assay Kit

User Manual

Catalog # FTA0139

(Version 1.2A)

Detection and Quantification of Phytase Activity in Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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 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. 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

III. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 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 (1 mmol/L)	1 ml x 1	-20 °C
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
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Note:

Substrate: add 8 ml Reaction Buffer into Substrate, mix.

Dye Reagent: add 5 ml Dye Reagent III into Dye Reagent I and 1 ml Dye Reagent III into Dye Reagent II respectively to dissolve. Transfer all Dye Reagent II into 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 solution is polluted. This solution should be prepared before use. It is best to use disposable plastic containers to prepare the solution in order to prevent phosphorus pollution.

Positive Control: add 1 ml distilled water to dissolve before use, then add 0.1 ml into 0.9 ml distilled water. Store at -20 °C.

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

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

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 20 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	Blank	Standard	Positive Control
Substrate	80 µl	80 µl	--	80 µl
Sample	20 µl	--	--	--
Distilled water	--	20 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	100 µl	100 µl	100 µl	100 µl
Mix, measured at 660 nm and record the absorbance.				

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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 sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

VI. CALCULATION

Unit Definition: One unit of phytase activity is defined as the enzyme the enzyme generates 1 μmol of PO_4^{3-} per minute.

1. According to the protein concentration of sample

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

$$= 0.5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= 0.5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cell or bacteria

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

$$= 0.5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

$$\text{Phytase (U/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 0.5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

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

$C_{Standard}$: the concentration of Standard, 1 mmol/L = 1 μmol/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

V_{Sample}: the volume of sample, 0.02 ml;

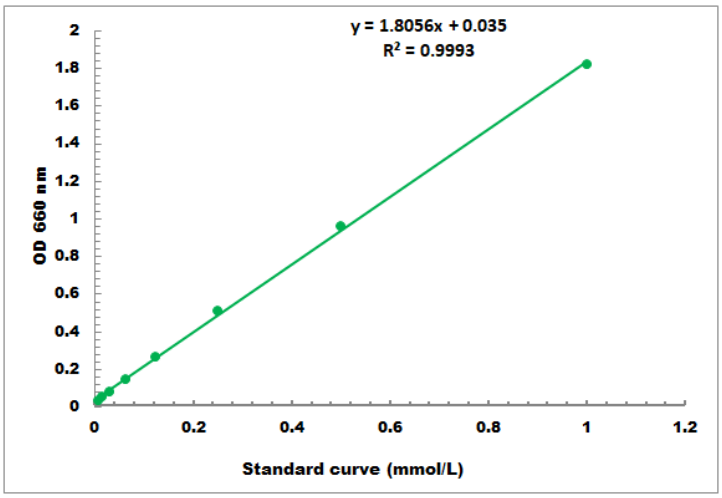
$V_{Standard}$: the volume of sample, 0.1 ml;

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

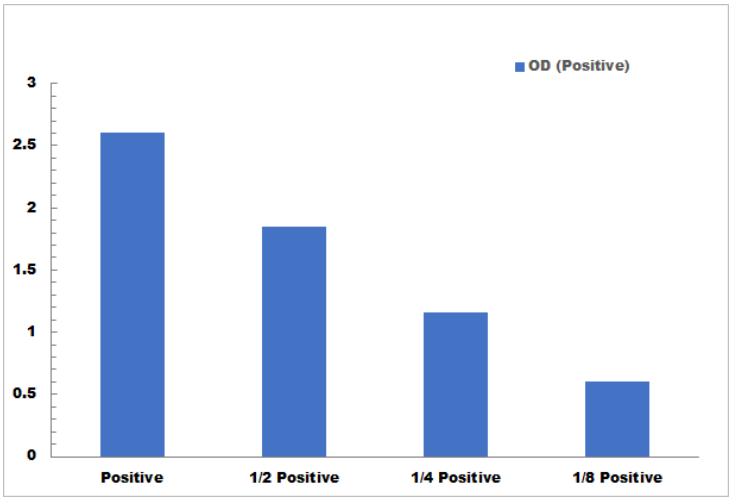
T: the reaction time, 10 minutes.

VII. TYPICAL DATA

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



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



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

VIII. TECHNICAL SUPPORT

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

IX. NOTES