Phytic Acid Microplate Assay Kit

User Manual

Catalog # CAK1224

(Version 1.2A)

Detection and Quantification of Phytic Acid Content 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

Phytic acid is a six-fold dihydrogenphosphate ester of inositol, also called inositol hexakisphosphate or inositol polyphosphate. At physiological pH, the phosphates are partially ionized, resulting in the phytate anion. The phytate anion is a colorless species that has significant nutritional role as the principal storage form of phosphorus in many plant tissues, especially bran and seeds. It is also present in many legumes, cereals, and grains.

Phytic Acid Microplate Assay Kit is designed to measure phytic acid content 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-Well Microplate	1 plate	
Reaction Buffer	8 ml x 1	4 °C
Enzyme	Powder x 1	-20 °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	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
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Note:

Enzyme: add 1.1 ml distilled water to dissolve before use, centrifuged at 4000g for 5 minutes, store at -20 °C.

Standard: add 1.25 ml distilled water to dissolve before use, the concentration will be 4 mmol/L.

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.

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. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml distilled water for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 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 distilled water 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.

3. For liquid samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Blank	Standard
Sample	10 µl	--	--
Distilled water	--	10 µl	--
Standard	--	--	10 µl
Reaction Buffer	80 µl	80 µl	80 µl
Enzyme	10 µl	10 µl	10 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
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) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

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

VI. CALCULATION

1. According to the protein concentration of sample

$$\text{Phytic Acid } (\mu mol/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein})$$

$$= 4 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$\text{Phytic Acid } (\mu mol/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay})$$

$$= 4 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cell or bacteria

$$\text{Phytic Acid } (\mu mol/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay})$$

$$= 4 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

$$\text{Phytic Acid } (\mu mol/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 4 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

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

$C_{Standard}$: the concentration of Standard, 4 mmol/L = 4 μmol/ml;

W: the weight of sample, g;

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

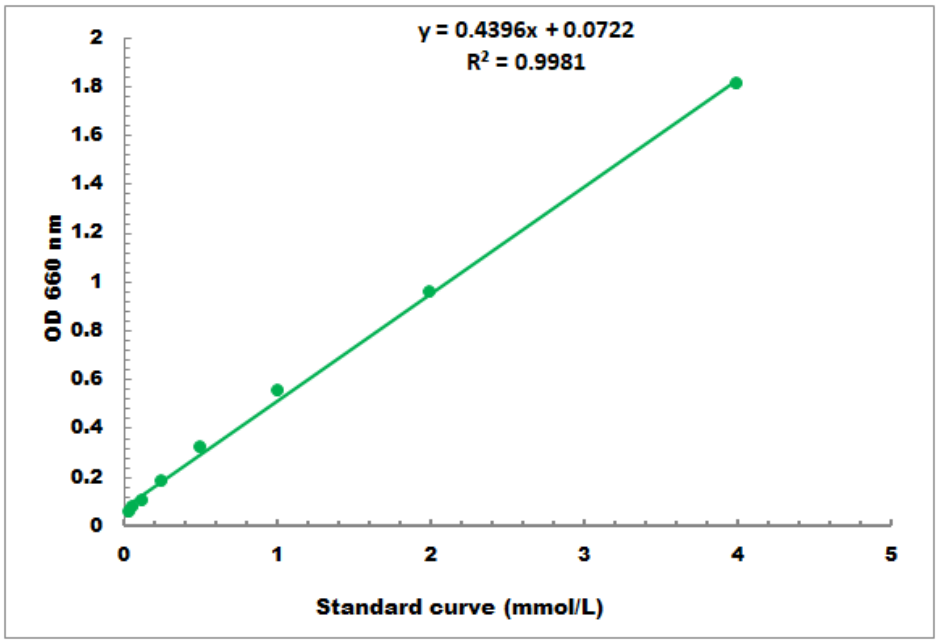
V_{Sample}: the volume of sample, 0.01 ml;

$V_{Standard}$: the volume of standard, 0.01 ml;

V_{Assay}: the volume of distilled water, 1 ml.

VII. TYPICAL DATA

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



Detection Range: 0.04 mmol/L - 4 mmol/L

VIII. TECHNICAL SUPPORT

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

IX. NOTES