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Phytic Acid

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1224

(Version 1.4A)

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

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 AcidColorimetric Microplate Assay Kit is designed to measure phytic acidcontentin 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	
Reaction Buffer	8 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Dye Reagent I	Powder x 1	4 °C, keep in dark
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	
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. Hot air circulation oven

IV. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1.25 mldistilled waterto generate 4 mmol/L of standardstock/top solution, store at 4 °C for 3-4 monthsafter reconstitution.Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be4/2/1/0.5/0.25/0.125/0.0625mmol/L.

Enzyme: Briefly centrifuge prior to opening. Add 1.1 ml distilled waterto dissolve before use, centrifuged at 4000g for 5minutes, transfer the supernatant and store at -20°C for 1 month.

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 -20 °C for 2-3 weeks.

***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.

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 mldistilled water for 5 $\times 10^6$ 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 mldistilled water on ice, centrifuged at 8000g 4°C for 20minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For liquid samples

Detect directly.

VI. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent*	Sample**	Blank	Standard
Reaction Buffer	80 µl	80 µl	80 µl
Sample	10 µl	--	--
Distilled water	--	10 µl	--
Standard	--	--	10 µl
Enzyme	10 µl	10 µl	10 µl
Mix, cover the plate adhesive strip, put the plate into the hot air circulation oven, incubate at 55 °C for 10 minutes.			
Dye Reagent	100µl	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.

**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.

**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

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

$$C=\frac{(OD_{Sample}-OD_{Blank})-\text{Intercept}}{\text{Slope}}\times n(mmol/L)$$

Calculate the initial concentration according to sample preparation procedure.

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

2.1According to the quantity of cells or bacteria

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

2.2 According to the weight of sample

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

2.3According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}}(\mu mol/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, mmol/L = μmol/ml

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

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

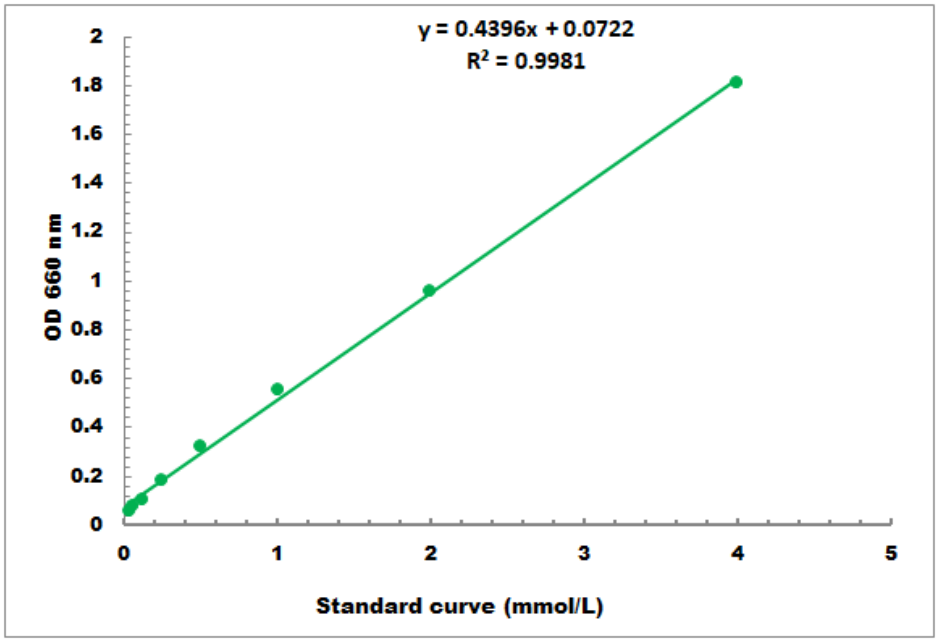
V_{Assay}: the volume of Assay Buffer, μl

W: the weight of sample, g

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

VIII. TYPICAL DATA

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



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