Phytic acid Assay Kit

ARG83416 Phytic acid Assay Kit can be used to measure Phytic acid in tissue extracts, cell lysate, cell culture media and other biological fluids.

Catalog number: ARG83416

Package: 96 wells

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

The (myo) 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 and phytate have a strong binding affinity to the dietary minerals, calcium, iron, and zinc, inhibiting their absorption in the small intestine.

PRINCIPLE OF THE ASSAY

The Phytic acid Assay Kit can measure Phytic acid in tissue extracts, cell lysate, cell culture media and other biological fluids. The increase in absorbance at 660 nm is directly proportional to reactants of the reaction between substrate and phytic acid.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard	1 vial (lyophilized)	4°C
Enzyme	1 vial (lyophilized)	-20°C
Reaction Buffer	8 ml (ready to use)	4°C
Reagent Dye I	1 vial (lyophilized)	4°C
Reagent Dye II	1 vial (lyophilized)	4°C
Reagent Dye III	10 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 660 nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store Enzyme at -20°C, all other components store at 4°C.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell and bacteria - Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml distilled water for 5 × 106 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.

Tissue - 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.

*Note: liquid samples can detect directly.

REAGENT PREPARATION

- **Standard:** Add **1.25 ml** of **distilled water** to dissolve standard. The concentration will be 4 mmol/L. Perform 2-fold serial dilution of the top standards to make the standard curve.
- **Enzyme:** Add **1.1 ml** of **distilled water** to dissolve Enzyme stored at -20°C.

- **Reagent Dye**: 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. This solution should be prepared before use.

  *Note: It should be yellow. If colorless, the solution is failure. If blue, the solution is polluted.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Sample wells: Add **10 µl Sample** into Sample wells.
2. Standard wells: Add **10 µl Standard** into Standard wells.
3. Add **80 µl Reaction Buffer** to each wells.
4. Add **10 µl Enzyme** to each wells.
5. Mix well. Incubate at **55°C** for **10 min**
6. Add **100 µl Reagent Dye** to each wells.
7. Mix well. Read the OD at **660nm**

Reagent	Sample	Standard	Blank
Sample	10 µl	-	-
Standard	-	10 µl	-
Distilled water	-		10 µl
Reagent Buffer	80 µl	80 µl	80 µl
Enzyme	10 µl	10 µl	10 µl
Mix well. Incubate at 55°C for 10 min			
Reagent Dye	100 µl	100 µl	100 µl
Mix well. Read the OD at 660nm			

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of samples, standard and blank.

2. Calculation:

A. Definition:

$C_{Standard}$: the standard concentration, 4 µmol /ml;

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

W: the weight of sample, g;

V_{Sample}: the volume of reaction sample, 10 µl = 0.01 ml;

$V_{standard}$: the volume of standard, 10 µl = 0.1 ml;

V_{assay}: the volume of Assay Buffer, 1000 µl = 1 ml.

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

B. Formula:

a). According to the protein concentration of sample

Phytic acid (μmol/g) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{blank})] / [(OD_{Standard} - OD_{Blank}) \times (C_{Protein} \times V_{Sample})]$

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

b). According to the weight of sample

Phytic acid (μmol/g) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{blank})] / [(OD_{Standard} - OD_{Blank}) \times (W \times V_{Sample} / V_{assay})]$

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

c). According to the volume of sample

Phytic acid (μmol/g) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{blank})] / [(OD_{Standard} - OD_{Blank}) \times V_{assay})]$

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

d). According to the cell or bacteria

Phytic acid (μmol /10^4 cell) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{blank})] / [(OD_{Standard} - OD_{Blank}) \times (N \times V_{Sample} / V_{Assay})]$

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

3. Detection range:

The detection range is from 0.04 mg/ml - 4mg/ml.

4. If the samples have been diluted, the calculated activity must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL RESULT

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. Please note this data is for demonstration only and serial diluted standards are not necessary for this kit.