（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K869-M

Specification：96T (40 samples)

Measuring instrument：Microplate reader (690-705 nm)

Detection range：3.78-500 nmol/mL

Elabscience® Phytic Acid Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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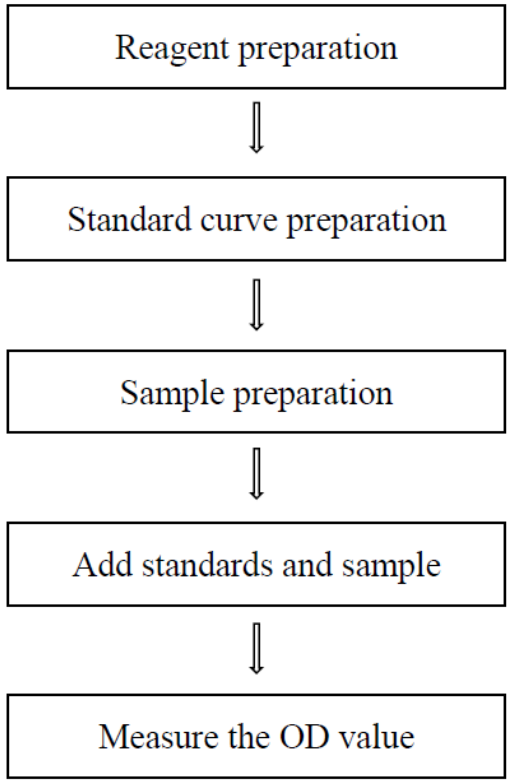
Assay summary



Intended use

This kit can be used to measure phytic acid content in plant tissue and liquid samples.

Detection principle

Phytic acid, also known as inositol hexaphosphate, is widely found in cereals, beans, and vegetables. The detection principle of this kit: Phytic acid generates inorganic phosphorus under the catalysis of enzyme. Under acidic conditions, inorganic phosphorus reacts with ammonium molybdate to produce blue molybdenum blue substance, which has a characteristic absorption peak at 700 nm. By measuring the content of inorganic phosphorus, the phytic acid content can be calculated.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution A	60 mL × 1 vial	2-8℃, 12 months
Reagent 2	Extraction Solution B	10 mL × 1 vial	2-8℃, 12 months
Reagent 3	Buffer Solution	20 mL × 1 vial	2-8℃, 12 months
Reagent 4	Chromogenic Agent A	Powder × 1 vial	-20℃, 12 months, shading light
Reagent 5	Chromogenic Agent B	Powder × 1 vial	2-8℃, 12 months
Reagent 6	Accelerant	Powder × 1 vial	2-8℃, 12 months
Reagent 7	Standard	Powder × 1 vial	2-8℃, 12 months
Reagent 8	Diluent Solution	15 mL × 1 vial	2-8℃, 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain

sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (690-705 nm, optimum wavelength: 700 nm)

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② The preparation of chromogenic A working solution:
Dissolve one vial of chromogenic agent A with 8 mL of buffer solution, mix well to dissolve. Aliquoted storage at -20 ℃ for 4 weeks protected from light, and avoid repeated freeze/thaw cycles is advised.

③ The preparation of chromogenic B working solution:
Dissolve one vial of chromogenic agent B with 15 mL of double distilled water, mix well to dissolve. Store at 2-8 ℃ for 4 weeks.

④ The preparation of accelerant working solution:
Dissolve one vial of accelerant with 15 mL of double distilled water, mix well to dissolve. Store at 2-8 ℃ for 4 weeks.

⑤ The preparation of chromogenic working solution:
For each well, prepare 150 μL of chromogenic working solution (mix well 50 μL of chromogenic B working solution, 50 μL of accelerant working solution and 50 μL of diluent solution). The chromogenic working solution should be prepared on spot.

⑥ The preparation of 10 μmol/mL standard solution:
Dissolve one vial of standard with 1.08 mL of buffer solution, mix well to dissolve. Store at 2-8 ℃ for 4 weeks.

⑦ The preparation of 1000 nmol/mL standard solution:
Before testing, please prepare sufficient 1000 nmol/mL standard solution. For

example, prepare 500 μL of 1000 nmol/mL standard solution (mix well 50 μL of 10 μmol/mL standard solution and 450 μL of double distilled water). The 1000 nmol/mL standard solution should be prepared on spot.

⑧ The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 1000 nmol/mL standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 50, 100, 200, 250, 300, 400, 500 nmol/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (nmol/mL)	**0**	**50**	**100**	**200**	**250**	**300**	**400**	**500**
1000 nmol/mL Standard (μL)	0	10	20	40	50	60	80	100
Double distilled water (μL)	200	190	180	160	150	140	120	100

Sample preparation

① Sample preparation

Liquid samples:

① Take 100 μL of samples and add 1 mL of extraction solution A. Oscillate at 25℃ for 2 h.

② Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Take 0.8 mL supernatant and add 0.15 mL of extraction solution B. Slowly blow and mix until no bubbles occur.

③ Centrifuge at 10000×g for 10 min at 4℃. Collect supernatant for detection.

Fresh plant tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 100 mg).

② Homogenize 100 mg tissue in 0.9 mL extraction solution A with a dounce homogenizer at 4℃. Oscillate at 25℃ for 2 h.

③ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Take 0.8 mL supernatant and add 0.15 mL of extraction solution B. Slowly blow and mix until no bubbles occur.

④ Centrifuge at 10000×g for 10 min at 4℃. Collect supernatant for detection.

Plant tissue powder sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 50 mg).

② Homogenize 50 mg tissue powder in 0.95 mL extraction solution A with a dounce homogenizer at 4℃. Oscillate at 25℃ for 2 h.

③ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Take 0.8 mL supernatant and add 0.15 mL of extraction solution B. Slowly blow and mix until no bubbles occur.

④ Centrifuge at 10000×g for 10 min at 4℃. Collect supernatant for detection.

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Tomato tissue homogenate	1
10% Carota tissue homogenate	1
10% Green pepper tissue homogenate	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① After add the extraction solution B slowly, will produce a large number of bubbles, so it is recommended to use 2 mL EP tube for operation.

② If the sample appears turbidity after adding the chromogenic working solution, it is recommended to dilute the sample supernatant with normal saline (0.9% NaCl) before measuring.

③ The extraction solution A contains a protein precipitation component, so the supernatant cannot be used for protein concentration determination. For determination of protein content, separate sample should be taken.

Operating steps

① Standard tube: Take 120 μL of different concentrations of standard solutions from standard tube to the corresponding tubes.
Sample tube: Take 120 μL of samples to the corresponding tubes.
Control tube: Take 120 μL of samples to the corresponding tubes.

② Add 50 μL of buffer solution to control tubes. Add 50 μL of chromogenic A working solution to standard and sample tubes.

③ Incubate at 37 ℃ for 30 min.

④ Add 150 μL of chromogenic working solution to each tube.

⑤ Stand at 25 ℃ for 10 min. Take 200 μL the supernatant of each tube to the microplate with a micropipette. Measure the OD value of each well at 700 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.
3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

Tissue samples:

$$\text{phytic acid content (nmol/g wet weight)} = (\Delta A_{700} - b) \div a \div \frac{m}{V_1} \div V_3 \times (V_3 + V_2) \times f$$

$$= (\Delta A_{700} - b) \div a \div m \div 0.842 \times f$$

or

$$\text{phytic acid content (nmol/mgprot)} = (\Delta A_{700} - b) \div a \div C_{pr} \div V_3 \times (V_3 + V_2) \times f$$

$$= (\Delta A_{700} - b) \div a \div C_{pr} \div 0.842 \times f$$

Liquid samples:

$$\text{phytic acid content (nmol/mL)} = (\Delta A_{700} - b) \div a \div V_3 \times (V_3 + V_2) \times f$$

$$= (\Delta A_{700} - b) \div a \div 0.842 \times f$$

[Note]

ΔA_{700}: $\Delta A_{700} = OD_{sample} - OD_{control}$.

m: The weight of sample, g.

V_1: The volume of extraction solution A, 1 mL.

V_2: The volume of extraction solution B, 0.15 mL.

V_3: The volume of supernatant after adding extraction solution A homogenate, 0.8 mL.

C_{pr}: Concentration of protein in sample, mgprot/mL.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three tomato tissue samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (nmol/mL)	150.00	250.00	400.00
%CV	4.1	3.2	2.3

Inter-assay Precision

Three tomato tissue tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (nmol/mL)	150.00	250.00	400.00
%CV	6.0	5.2	4.5

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 100.7%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (nmol/mL)	150.00	250.00	400.00
Observed Conc. (nmol/mL)	144.00	255.00	416.00
Recovery rate (%)	96.0	102.0	104.0

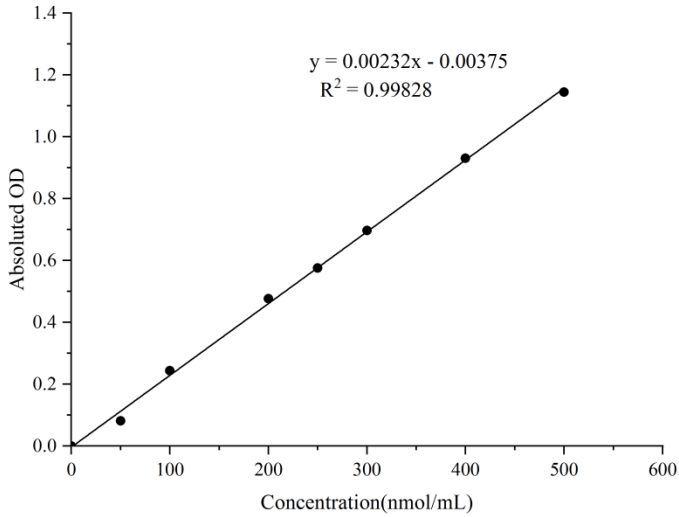
Sensitivity

The analytical sensitivity of the assay is 3.78 nmol/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (nmol/mL)	**0**	**50**	**100**	**200**	**250**	**300**	**400**	**500**
OD	0.163	0.241	0.419	0.634	0.730	0.847	1.078	1.290
	0.158	0.243	0.389	0.640	0.742	0.868	1.104	1.319
Average OD	0.160	0.242	0.404	0.637	0.736	0.858	1.091	1.305
Absolute OD	0.000	0.082	0.244	0.477	0.576	0.698	0.931	1.145



Appendix II Example Analysis

Example analysis:

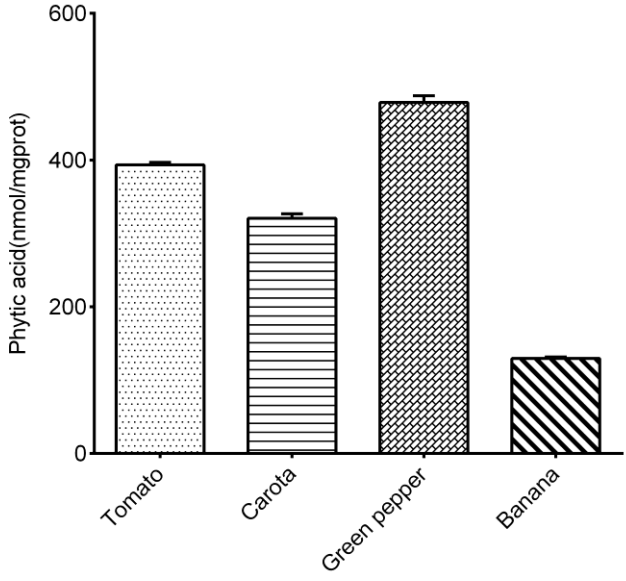
Take 0.1 g of tomato tissue and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.00232 x – 0.00375, the OD value of the sample well is 0.747, the OD value of the control well is 0.695, the concentration of protein is 0.072 mgprot/mL, and the calculation result is:

$$\text{phytic acid content (nmol/mgprot)} = (0.747 - 0.695 + 0.00375) \div 0.00232 \div 0.842 \div 0.072$$

$$= 396 \text{ nmol/mgprot}$$

Detect 10% tomato tissue homogenate (the concentration of protein is 0.072 mgprot/mL), 10% carota tissue homogenate (the concentration of protein is 0.031 mgprot/mL), 10% green pepper tissue homogenate (the concentration of protein is 0.024 mgprot/mL) and 10% banana tissue homogenate (the concentration of protein is 0.025 mgprot/mL), according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.