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

Catalog No：E-BC-K1210-M

Specification：96T (40 samples)

Measuring instrument：Microplate reader(650-670 nm)

Detection range：6.05-500 μmol/L

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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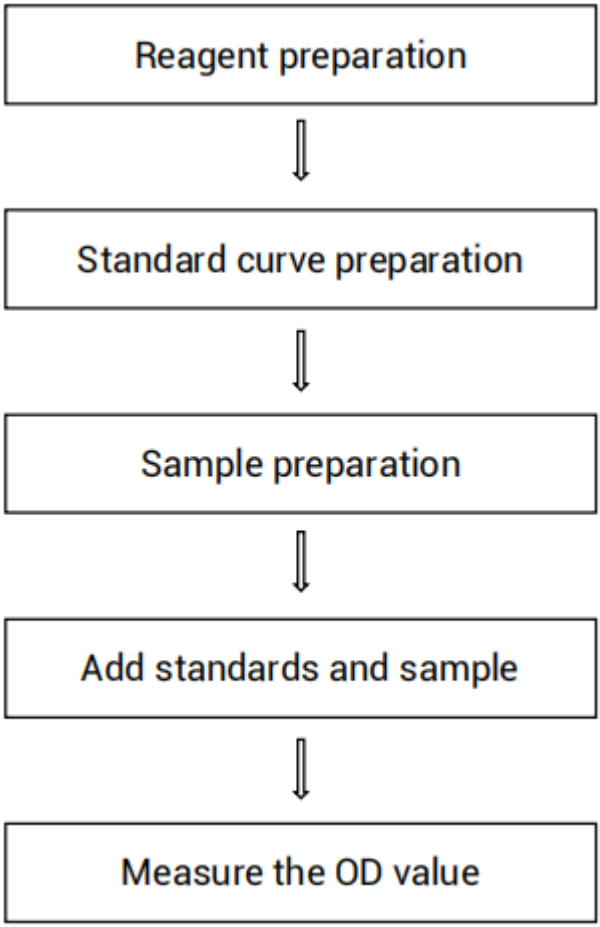
Assay summary



Intended use

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

Detection principle

Phytic Acid is the main source of inositol and stored phosphorus in plant seeds, accounting for up to 90% of the total phosphorus.
The detection principle of this kit: Enzymes can decompose phytic acid to produce inorganic phosphorus and inositol derivatives. The generated inorganic phosphorus reacts with the molybdate ammonium chromogenic agent, and the increase in OD value at 660 nm can be used to calculate the content of phytic acid.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution	48 mL × 1 vial	-20°C, 12 months, shading light
Reagent 2	Matrix Solution	7 mL × 1 vial	-20°C, 12 months, shading light
Reagent 3	Buffer Solution	38 mL × 1 vial	-20°C, 12 months, shading light
Reagent 4	Enzyme Reagent	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 5	Chromogenic Agent A	Powder × 1 vial	-20°C, 12 months, shading light
Reagent 6	Chromogenic Agent B	11 mL × 1 vial	-20°C, 12 months, shading light
Reagent 7	Chromogenic Agent C	11 mL × 1 vial	-20°C, 12 months, shading light

Reagent 8	Stop Solution	11 mL × 1 vial	-20°C, 12 months, shading light
Reagent 9	10 mmol/L Standard Solution	0.1 mL × 1 vial	-20°C, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

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 (650-670 nm, optimum wavelength: 660 nm), Incubator,

Reagent:

Double distilled water

Reagent preparation

① Keep extraction solution and matrix solution on incubator at 37°C before use. Equilibrate other reagents to 25°C before use.

② The preparation of enzyme working solution：

Dissolve one vial of enzyme reagent with 5 mL of buffer solution, mix well to dissolve (It is a normal phenomenon for solutions to contain insoluble substances). Centrifuge at 500 × g at 4 °C for 5 min and take the supernatant on ice for use. Aliquoted storage at -20°C for 7 days protected from light.

③ The preparation of chromogenic agent A working solution：

Dissolve one vial of chromogenic agent A with 11 mL of double distilled water, mix well to dissolve. Aliquoted storage at 2-8°C for 14 days protected from light.

④ The preparation of chromogenic working solution：

For each well, prepare 300 μL of chromogenic working solution (mix well 100 μL of chromogenic agent A working solution, 100 μL of chromogenic agent B and 100 μL of chromogenic agent C, yellow transparent liquid). Store for 1 day protected from light.

⑤ The preparation of 500 μmol/L standard solution：

Before testing, please prepare sufficient 500 μmol/L standard solution according to the test wells. For example, prepare 2000 μL of 500 μmol/L standard (mix well 100 μL of 10 mmol/L standard solution and 1900 μL of buffer solution). Aliquoted storage at -20°C for 7 days protected from light.

⑥ The preparation of standard curve：

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

Dilute 500 μmol/L standard solution with buffer solution to a serial concentration, the recommended dilution gradient is as follows: 0, 100, 150, 200, 250, 300, 350, 500 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑦	⑧	⑨
Concentration (μmol/L)	**0**	**100**	**150**	**200**	**250**	**300**	**350**	**500**
500 μmol/L standard (μL)	0	40	60	80	100	120	140	200
Buffer solution (μL)	200	160	140	120	100	80	60	0

Sample preparation

① Sample preparation

Liquid samples: detect directly.

Tissue samples:

① Harvest the amount of tissue needed for each assay (initial recommendation 30 mg).

② Homogenize 20 mg tissue in 270 μL extraction solution with a dounce homogenizer at 4℃.

③ Stir the homogenate at 1000 rpm at room temperature for 90 min. Centrifuge at 10000 × g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

④ Prepare 330 μL of solution (mix well 200 μL of supernatant, 30 μL of matrix solution and 100 μL of buffer solution). Mix slowly (be careful to avoid bubble formation). Stand for 10 min before use, detect within 4 h.

② Dilution of sample

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

Sample type	Dilution factor
10% Cinnamon leave tissue homogenate	1
10% Scindapsus aureus leaf tissue homogenate	1
10% Horse chestnut leaf tissue homogenate	1
10% Heath leave tissue homogenate	1
10% Ligustrum leaf tissue homogenate	1
10% Small wax leave tissue homogenate	1

Note: The diluent is buffer solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① The experimental equipment used needs to be rinsed several times (about 10 times) to avoid external phosphorus contamination.

② Try to avoid touching the mouths of containers holding the chromogenic agaent and the chromogenic working solution with the experimental gloves to prevent phosphorus contamination that could cause the chromogenic agent to color.

③ Extraction solution and matrix solution will precipitate at low temperatures, and be heated to 37°C to dissolve completely.

Operating steps

① Standard tube: Take 100 μL of standard solution with different concentrations into the 1.5 mL EP tubes.
Sample tube: Take 100 μL of sample into the 1.5 mL EP tubes.
Control tube: Take 100 μL of sample into the 1.5 mL EP tubes.

② Add 100 μL of enzyme working solution into standard tubes and sample tubes.
Add 100 μL of buffer solution into control tubes.

③ Centrifuge at 500 × g for 1 min.

④ Incubate at 37°C for 1 h protected from light.

⑤ Add 100 μL of stop solution into each tube.

⑥ Add 300 μL of chromogenic working solution into each tube.

⑦ Centrifuge at 500 × g for 1 min.

⑧ Incubate at 37°C for 15 min protected from light.

⑨ Take 200 μL of solution into corresponding wells, mix fully for 5 s with microplate reader. Measure the OD value of each well at 660 nm.

Calculation

① **The liquide sample:**

$$\text{Phytic Acid content (μmol/L)} = (OD_{sample} - OD_{control} - b) \div a \times f \times 1.65*$$

② **The tissue sample**:

$$\text{Phytic Acid content (μmol/kg wet weight)} = (OD_{sample} - OD_{control} - b) \div a \times V \div m \times f \times 1.65*$$

[Note]

OD_{sample}: The OD value of sample well

$OD_{control}$: The OD value of control well

m: The weight of tissue sample, g

V: The volume of homogenate, mL

1.65*: Dilution factor of sample processing procedure

f: Dilution factor of sample before tested

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three 10% heath leave tissue homogenate 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 (μmol/L)	150.0	200.0	300.0
%CV	1.2	2.4	1.8

Inter-assay Precision

Three 10% heath leave tissue homogenate samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	150.0	200.0	300.0
%CV	3.5	4.8	3.8

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 99%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (μmol/L)	150.0	200.0	300.0
Observed Conc. (μmol/L)	147.0	200.0	297.0
Recovery rate (%)	98	100	99

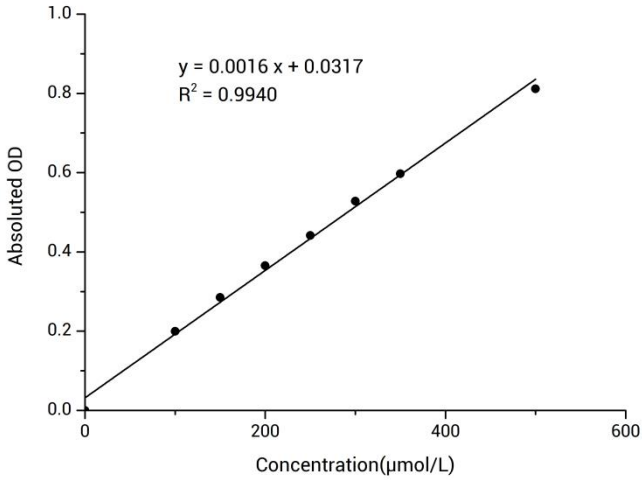
Sensitivity

The analytical sensitivity of the assay is 6.05 μmol/L. 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 (μmol/L)	0	100	150	200	250	300	350	500
OD value	0.107	0.308	0.391	0.462	0.539	0.620	0.699	0.896
	0.118	0.316	0.404	0.494	0.569	0.661	0.720	0.952
Average OD value	0.113	0.312	0.398	0.478	0.554	0.641	0.710	0.924
Absolute OD value	0.000	0.200	0.285	0.366	0.442	0.528	0.597	0.812



Appendix Π Example Analysis

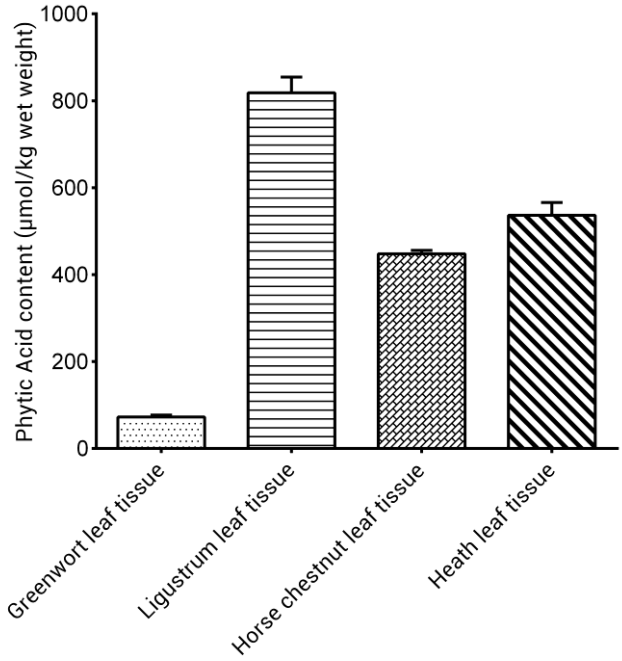
Example analysis：

Take 100 μL of 10% scindapsus aureus leaf tissue homogenate and carry the assay according to the opera tion table. The results are as follows: standard curve: y = 0.0016 x + 0.0317,the OD value of the sample well is 0.417, the OD value of the control well is 0.377, and the calculation result is:

Phytic Acid content（μmol/kg wet weight） = （0.417 - 0.377 - 0.0317） ÷ 0.0016 × 0.9 ÷ 0.1 × 1.65

= 77.05 μmol/kg wet weight

Detect 10% scindapsus aureus leaf tissue homogenate, 10% ligustrum leaf tissue homogenate, 10% horse chestnut leaf tissue homogenate, 10% heath leave tissue homogenate, 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.