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

Catalog No：E-BC-K1211-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader(650-670 nm)

Detection range：0.001-0.15 U/mL

Elabscience® Phytase Activity 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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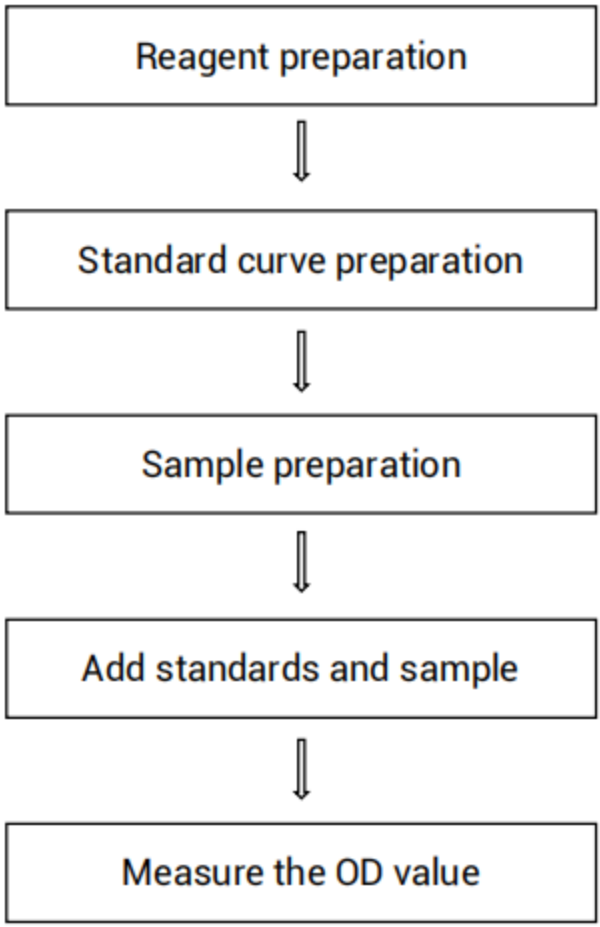
Assay summary



Intended use

This kit can be used to measure phytase activity in plant tissue samples.

Detection principle

Phytase is the general term for a class of enzymes that catalyze the hydrolysis of phytic acid and its salts into inositol and phosphate. It belongs to the category of phosphate monoester hydrolases. Phytase has a special spatial structure and can sequentially separate the phosphorus in phytic acid molecules, converting phytic acid (salt) into inositol and inorganic phosphorus, while releasing other nutrients bound to phytic acid. The detection principle of this kit: Phytase can decompose the substrate to produce inorganic phosphorus. Under acidic conditions, inorganic phosphorus reacts with the molybdate ammonium chromogenic agent to produce blue molybdenum blue substance. The content of inorganic phosphorus can be detected at a wavelength of 660 nm to calculate the activity of phytase.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution	40 mL × 2 vials	-20℃, 12 months
Reagent 2	Buffer Solution	12 mL × 1 vial	-20℃, 12 months, shading light
Reagent 3	Substrate	0.75 mL × 1 vial	-20℃, 12 months, shading light
Reagent 4	Chromogenic Agent A	Powder × 1 vial	-20℃, 12 months, shading light
Reagent 5	Chromogenic Agent B	11 mL × 1 vial	-20℃, 12 months, shading light
Reagent 6	Chromogenic Agent C	11 mL × 1 vial	-20℃, 12 months, shading light

Reagent 7	Stop Solution	11 mL × 1 vial	-20℃, 12 months, shading light
Reagent 8	1 mmol/L Standard Solution	1.6 mL × 2 vials	-20℃, 12 months, shading light
	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 (650-670 nm, optimum wavelength: 660 nm), Incubator

Reagent preparation

① Equilibrate all the reagents to 25°C before use. If chromogenic agent A and chromogenic agent B precipitate, heat at 37°C until completely dissolved before use.

② The preparation of substrate working solution：
For each well, prepare 100 μL of substrate working solution (mix well 10 μL of substrate and 90 μL of buffer solution). The prepared solution should be used up within 8 h 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. Store at 2-8°C for 15 days protected from light.

④ The preparation of chromogenic working solution：

Before testing, please prepare sufficient chromogenic working solution according to the test wells. For example, 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, transparent yellow is effective). Store at 25 °C for 1 day protected from light.

⑤ The preparation of 1 mmol/L standard solution：

The preparation of standard curve：

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

Dilute 1 mmol/L standard solution with double distilled water to a serial concentration, the recommended dilution gradient is as follows: 0, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7,1 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**1**
1 mmol/L standard (μL)	0	40	60	80	100	120	140	200
Double distilled water (μL)	200	160	140	120	100	80	60	0

Sample preparation

① Sample preparation

Tissue samples:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Homogenize 20 mg tissue in 180 μL extraction solution with a dounce homogenizer at 4 °C.

③ Centrifuge at 10000 × g for 10 min at 4 °C to remove insoluble material. Collect supernatant and keep it on ice for detection and 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% Spinach tissue homogenate	1
10% Bean Sprouts tissue homogenate	1
10% Broccoli tissue homogenate	1
10% Pumpkin tissue homogenate	1
10% Chive tissue homogenate	1

Note: The diluent is extraction 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 container used needs to be rinsed several times (about 10 times) with double distilled water to avoid external phosphorus contamination.

② The laboratory gloves should try to avoid touching the mouths of containers holding the chromogenic agent and the chromogenic working solution to prevent phosphorus contamination from causing false positive results.

Operating steps

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

③ Add 100 μL of substrate working solution into standard tubes and sample tubes.
Add 100 μL of buffer solution into control tubes.

④ Centrifuge at 500×g for 1 min，incubate at 37 °C for 1 h protect from light.

⑤ Add 100 μL of stop solution to each tube.

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

⑦ Centrifuge at 500×g for 1 min，incubate at 37 °C for 10 min protect from light.

⑧ Take 200 μL of supernatant into microplate and mix fully for 5 s with microplate reader. Measure the OD values of each well at 660 nm with microplate reader.

Calculation

The tissue sample:

Definition: The amount of enzyme in 1 kg tissue per 1 h that catalyze substrate to product 1 mmol phosphate at 37°C is defined as 1 unit.

$$\text{phytase activity (U/kg wet weight)} = (OD_{sample} - OD_{control}) \div a \div T \times f \div \frac{m}{V}$$

[Note]

m: The weight of sample, g

V: The volume of extraction solution during sample processing, mL

f: Dilution factor of sample before tested.

T: Reaction time,1 h

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three 10% spinach 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 (U/L)	0.54	1.07	2.14
%CV	3.6	3.7	4.5

Inter-assay Precision

Three s10% spinach 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 (U/L)	0.54	1.07	2.14
%CV	5.7	4.8	7.2

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 100.3%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	0.06	0.12	0.24
Observed Conc. (U/L)	0.0588	0.1200	0.2472
Recovery rate (%)	98	100	103

Sensitivity

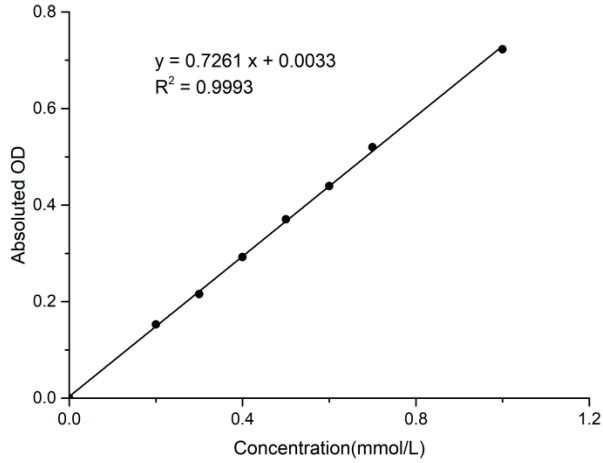
The analytical sensitivity of the assay is 0.001 U/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

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 (mmol/L)	0	0.2	0.3	0.4	0.5	0.6	0.7	1.0
OD	0.075	0.229	0.293	0.365	0.442	0.504	0.594	0.800
	0.075	0.227	0.289	0.371	0.450	0.526	0.597	0.796
Average OD	0.075	0.228	0.291	0.368	0.446	0.515	0.596	0.798
Absoluted OD	0.000	0.153	0.216	0.293	0.371	0.440	0.520	0.723



Appendix Π Example Analysis

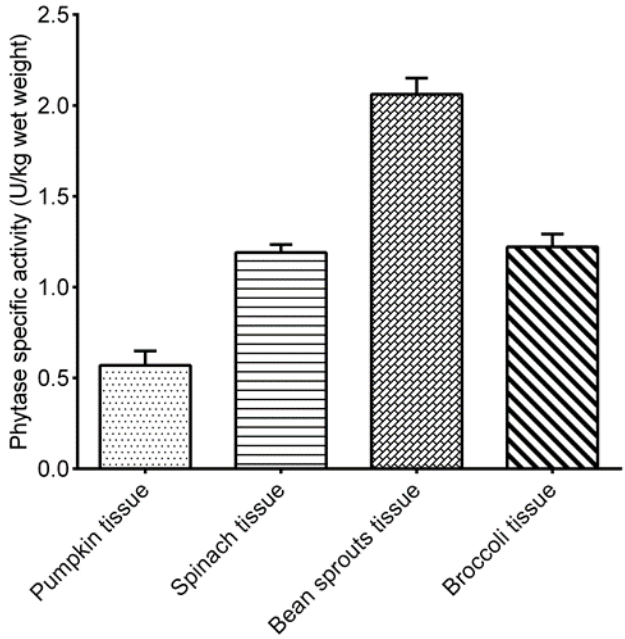
Example analysis：

Take 100 μL of 10% bean sprouts tissue homogenate and carry the assay according to the operation table. The results are as follows:

The standard curve: y = 0.7261 x + 0.0033, OD value of the sample well is 0.575, the OD value of the control well is 0.405, and the calculation result is:

$$\text{phytase activity (U/kg wet weight)} = (0.575-0.405-0.0033) \div 0.7261 \times 1 \div \frac{0.1}{0.9} = 2.066 \text{ U/kg wet weight}$$

Detect 10% pumpkin tissue homogenate, 10% spinach tissue homogenate, 10% bean sprouts tissue homogenate, 10% broccoli tissue homogenate 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.