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

Catalog No：E-BC-K870-M

Specification：96T(46 samples)

Measuring instrument：Microplate reader（690-710 nm）

Detection range：1-170 U/mL

Elabscience® Phytase Activity Colorimetric Assay Kit

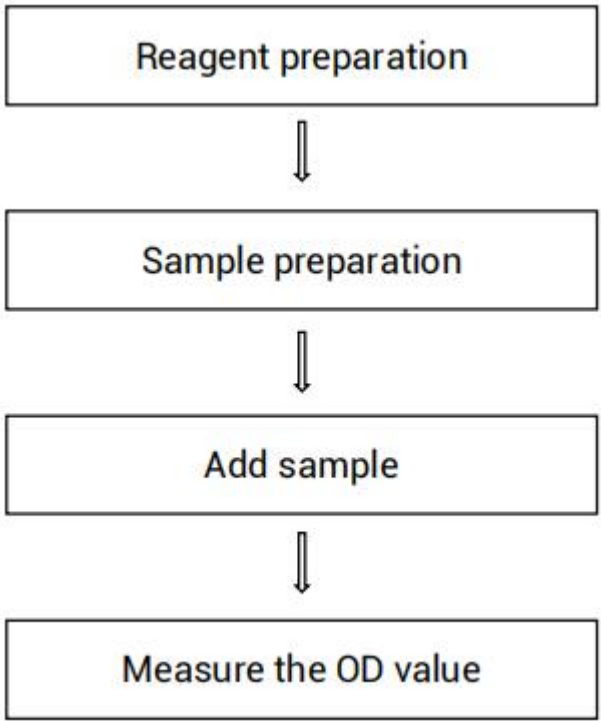
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

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.

Table of contents

Assay summary



Intended use

This kit can be used to measure phytase activity in plant tissue, animal tissue and microorganism samples.

Detection principle

Phytase also known as inositol hexaphosphatase, is a protein and sugar binding enzyme. Phytase can catabolize phytic acid to produce inorganic phosphorus and inositol, which greatly improves nutrient utilization rate of organisms. Natural phytase is widely found in plants, animal tissues and microorganisms. Phytase has a wide research value in the field of food production and animal husbandry.

Under certain environmental conditions, phytase can decompose sodium phytate to produce inorganic phosphorus and inositol derivatives. Under acidic conditions, inorganic phosphorus and ammonium molybdate chromogenic agent react to produce a blue molybdenum blue substance, which has a characteristic absorption peak at 700 nm. Phytase activity can be calculated by measuring the content of inorganic phosphorus.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extracting Solution	30 mL × 1 vial	2-8℃, 12 months
Reagent 2	Buffer Solution A	30 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 3	Substrate	Powder × 1 vial	2-8℃, 12 months, shading light
Reagent 4	Accelerant	Powder × 1 vial	2-8℃, 12 months, shading light
Reagent 5	Chromogenic Agent	Powder × 1 vial	2-8℃, 12 months, shading light

Reagent 6	10 μmol/mL Standard Solution	1 mL × 1 vial	2-8℃, 12 months
Reagent 7	Buffer Solution B	15 mL × 1 vial	2-8℃, 12 months
	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 (690-710 nm, optimum wavelength: 700 nm), Water bath

Reagents:

Concentrated sulfuric acid (98%)

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② The preparation of substrate working solution：
Dissolve one vial of substrate with 30 mL of buffer solution A (buffer solution A can be used to aspirate and wash substrate vial repeatedly), mix well to dissolve. Aliquoted storage at 2-8°C for 4 weeks.

③ The preparation of accelerant working solution：
Dissolve one vial of accelerant with 15 mL of double distilled water, mix well to dissolve. Aliquoted storage at 2-8°C for 4 weeks.

④ The preparation of chromogenic working solution：
Dissolve one vial of chromogenic agent with 15 mL of double distilled water, mix well to dissolve. Aliquoted storage at 2-8°C for 4 weeks.

⑤ The preparation of reaction working solution：
For each well, prepare 150 μL of reaction working solution (mix well 50 μL of accelerant working solution, 50 μL of chromogenic working solution and 50 μL of buffer solution B). Aliquoted storage at 2-8°C for 3 days.

⑥ The preparation of 1 μmol/mL standard solution：
For each well, prepare 50 μL of 1 μmol/mL standard solution (mix well 5 μL of 10 μmol/mL standard solution and 45 μL of double distilled water). The 1 μmol/mL standard solution should be prepared on spot and the solution should be used up within 1 day.

Sample preparation

① Sample preparation

Culture or other liquid samples: Test directly. If it is cloudy, centrifuge at 10000×g for 10 min at 4 ℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

Tissue samples:

① Harvest the amount of tissue needed for each assay (initial recommendation 0.05 g).

② Homogenize 0.05 g tissue in 0.5 mL extracting solution with a dounce homogenizer at 4℃.

③ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material (If it is still cloudy, the centrifugation time can be extended). Collect supernatant and keep it on ice for detection.

④ Meanwhile, determine the protein concentration of supernatant (Animal tissue: E-BC-K318-M; Plant tissue: E-BC-K168-M).

Cell or bacteria samples:

① Harvest the number of cell or bacteria needed for each assay (initial recommendation 2.5×10^6 cell or bacteria).

② Homogenize 2.5×10^6 cell or bacteria in 0.5 mL extracting solution) with a ultrasonic cell disruptor at 4℃. Ultrasonic disruption of bacteria or cells (ice bath, power 200 W, ultrasonic 3 s, interval 7 s, total time 3 min).

③ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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

Sample type	Dilution factor
10% barley tissue homogenate	1
10% carota tissue homogenate	1
10% mouse heart tissue homogenate	1
10% mouse liver tissue homogenate	1

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

The key points of the assay

① To prevent water loss during a 10-min boiling water bath, it is recommended to use a spiral-mouth EP tube or wrap the EP tube mouth with a sealing film.

② If the OD value of the sample is too low or close to blank, the reaction time of 37℃ water bath for 30 min in the second step should be appropriately extended or the volume of sample should be increased before determination.

Operating steps

① Sample tube: Take 50 μL of sample into 1.5 mL EP tube.
Control tube: Take 50 μL of sample into 1.5 mL EP tube.
Standard tube: Take 50 μL of 1 μmol/mL standard solution into 1.5 mL EP tube.

② Sample tube: Water bath in 37℃ for 5 min.
Control tube: Water bath in 37℃ for 5 min.

③ Add 120 μL of substrate working solution into sample tubes.

④ Sample tube: Water bath in 37℃ for 30 min, boiled water bath for 10 min.
Control tube: Water bath in 37℃ for 30 min, boiled water bath for 10 min.

⑤ Control tube: Add 120 μL of substrate working solution into control tubes.
Standard tube: Add 120 μL of double distilled water into standard tubes.
Blank tube: Add 170 μL of double distilled water into blank tubes.

⑥ Add 150 μL of reaction working solution to each tube.

⑦ Stand for 10 min in 25℃, centrifuge at 8000×g for 10 min at 25℃.Take 200 μL of supernatant into microplate and measure the OD values of each well at 700 nm with microplate reader.

Calculation

① **The tissue, cell and bacteria sample**:

Definition: The amount of enzyme in 1 mg tissue, cell or bacteria protein per 1 min that produce 1 nmol product at 37°C is defined as 1 unit.

$$\text{phytase activity U/mgprot} = \frac{\Delta A_{700}}{OD_{Standard} - OD_{Blank}} \div T \times f \times 1000* \div C_{pr}$$

② **The liquid sample:**

Definition:

The amount of enzyme in 1 mL liquid, per 1 min that produce 1 nmol product at 37°C is defined as 1 unit.

$$\text{phytase activity U/mL} = \frac{\Delta A_{700}}{OD_{Standard} - OD_{Blank}} \div T \times 1000* \times f$$

[Note]

ΔA_{700}: $OD_{Sample} - OD_{Control}$

T: Reaction time, 30 min.

f: Dilution factor of sample before tested.

1000*:1 μmol/mL=1000 nmol/mL

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

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three barley 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 (U/mL)	30.00	80.00	150.00
%CV	4.5	3.6	3.2

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	30.00	80.00	150.00
%CV	5.0	4.0	3.6

Sensitivity

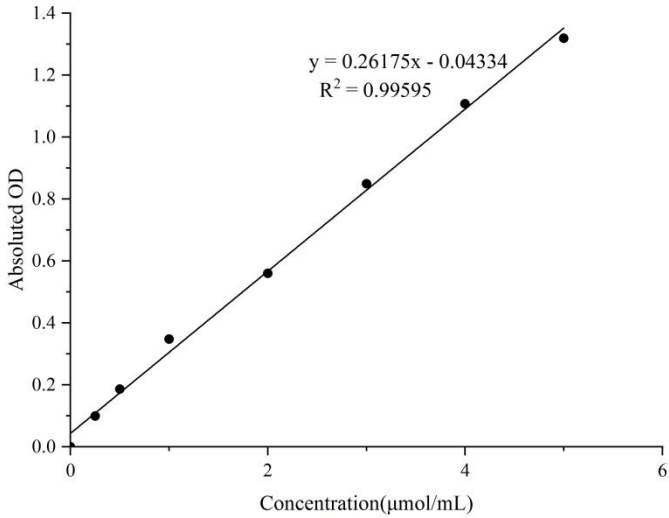
The analytical sensitivity of the assay is 1 U/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

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 (μmol/mL)	0	0.25	0.5	1	2	3	4	5
OD	0.074	0.174	0.260	0.419	0.633	0.921	1.179	1.395
	0.074	0.171	0.260	0.425	0.635	0.924	1.184	1.391
Average OD	0.074	0.173	0.260	0.422	0.634	0.923	1.181	1.393
Absoluted OD	0.000	0.099	0.186	0.348	0.560	0.849	1.108	1.319



Appendix Π Example Analysis

Example analysis：

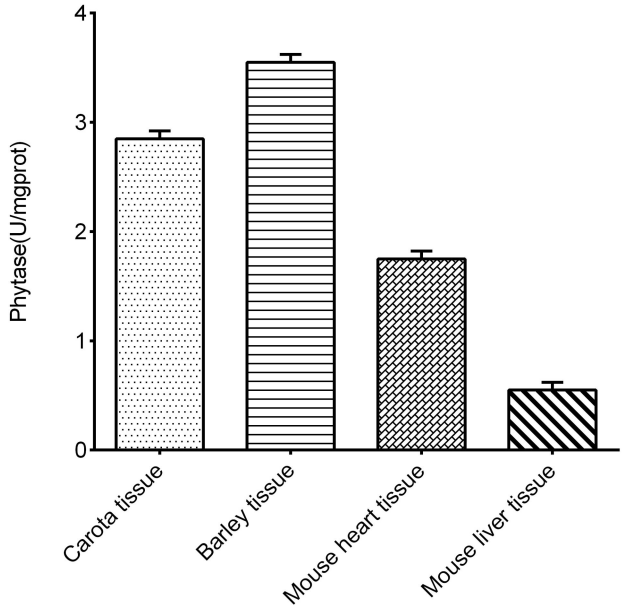
Take 0.05 g carota tissue and carry the assay according to the operation table. The results are as follows:

The OD value of the sample well is 0.086, the OD value of the control well is 0.058, the OD value of the blank well is 0.049, the OD value of the standard well is 0.348, the concentration of protein is 1.044 mgprot/mL, and the calculation result is:

$$\text{phytase activity (U/mgprot)} = (0.086 - 0.058) \div (0.348 - 0.049) \div 30 \div 1.044 \times 1000$$

$$= 2.98 \text{ U/mgprot}$$

Detect 10% carota tissue homogenate(the concentration of protein is 1.044 mgprot/mL), 10% barley tissue homogenate(the concentration of protein is 1.845 mgprot/mL), 10% mouse heart homogenate(the concentration of protein is 5.140 mgprot/mL), 10% mouse liver homogenate(the concentration of protein is 12.051 mgprot/mL) according to the protocol, the result is as follows：


Phytase(U/mgprot)
4
3
2
1
0
Carola tissue
Barley tissue
Mouse heart tissue
Mouse liver tissue

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.