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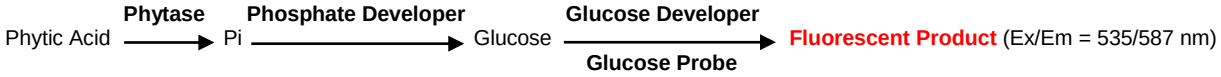
Phytase Activity Assay Kit (Fluorometric)

08/20

(Catalog # K2054-100; 100 assays; Store at -20°C)

I. Introduction:

Phytase belongs to the phosphatase enzyme family that hydrolyzes phytic acid, an indigestible form of phosphorous to inositol and free inorganic phosphorous. Phytic acid is often referred to as an anti-nutrient because it binds to minerals such as calcium, iron, zinc, potassium, magnesium in the digestive tract and reduces its absorption thereby leading to bone loss and toxic build-up. Phytase is produced by bacteria in the gut of ruminant animals such as cattle, sheep etc. making it possible for them to use the phytic acid found in grains, plant tissues as a source of phosphorus. However, animals such as dogs, pigs do not produce phytase and thus, their feed is supplemented with phytase. Phytase can reduce the anti-nutritional effect of phytate and improve the digestibility of phosphorous, calcium, amino acids, carbohydrates etc. Phytases can also be used as a human supplement to boost bone health and reduce osteoporosis. **BioVision's Phytase Activity Assay Kit** provides a facile, rapid way to monitor phytase activity in various biological samples. In this kit, phytase cleaves the phytic acid substrate and releases phosphate, which is then used to release glucose from a disaccharide. In subsequent reactions, glucose reacts with the probe and the developer to generate a fluorescent product measured at Ex/Em = 535/587 nm. The assay is simple, sensitive, and can detect as low as 1 µU of phytase activity under assay conditions.



II. Application:

- Measurement of phytase activity in various samples

III. Sample Types:

- Grains: Barley seeds, etc.
- Fungus: Mushrooms, etc.

IV. Kit Contents:

Components	K2054-100	Cap Code	Part Number
Phytase Assay Buffer	25 ml	WM	K2054-100-1
Phytase Substrate	2 x 1.5 ml	Amber	K2054-100-2
Phosphate Developer A	1 vial	Orange	K2054-100-3
Phosphate Developer B	50 µl	Blue	K2054-100-4
Glucose Probe	200 µl	Red	K2054-100-5
Glucose Developer	1 vial	Green	K2054-100-6
Glucose Standard	100 µl	Yellow	K2054-100-7
Phytase Positive Control	50 µl	Violet	K2054-100-8

V. User Supplied Reagents and Equipment:

- Multi-well spectrophotometer (plate reader)
- 96-Well Black Plates (BioVision Cat# M1355)
- Dounce Tissue Homogenizer (BioVision Cat# 1998)
- 10 kDa Spin Column (BioVision Cat# 1997)
- Glycerol (50%), Sterile Solution (BioVision Cat# B1012)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge all small vials before opening. Read the entire protocol before performing the assay. Upon opening, use it within two months.

- **Phytase Assay Buffer:** Warm to room temperature (RT) before use. Store at 4°C or -20°C.
- **Phytase Substrate and Glucose Standard:** Ready to use. Thaw at RT. Store at -20°C.
- **Phosphate Developer A & Glucose Developer:** To each vial, add 220 µl Phytase Assay Buffer. Divide into aliquots and store at -20°C. Keep on ice while in use. Avoid multiple free-thaw cycles.
- **Phosphate Developer B:** Add 450 µl of 50% Glycerol (not included) to the vial to prepare the Phosphate Developer B solution. Vortex to mix. Divide into aliquots and store at -20°C. Avoid multiple freeze-thaw cycles.
- **Glucose Probe:** Thaw at RT before use. Store at -20°C, protected from light.
- **Phytase Positive Control:** Divide into aliquots & store at -20°C. Always keep on ice when in use. Avoid multiple free-thaw cycles.

VII. Phytase Activity Assay Protocol:

1. Sample Preparation: For Grains or Fungus: Weigh out 10-30 mg of the sample, cut into small pieces, if needed. Transfer sample into an eppendorf tube and homogenize in 100-200 µl ice-cold Phytase Assay Buffer using Dounce Tissue Homogenizer (BioVision Cat# 1998). Keep on ice for 10-15 min. Centrifuge at 12,000 x *g* and 4°C for 15 min and collect the supernatant. To remove interference from endogenous inorganic phosphorous or glucose, dilute the lysate 5-10 fold with Phytase Assay Buffer and filter through 10 kDa Spin Column (BioVision Cat# 1997). Centrifuge at 10,000 x *g* and 4°C for 10 min and discard the filtrate. Adjust the ultra-concentrate to the original volume using Phytase Assay Buffer and repeat the procedure 3-4 times. Prepare two wells for each sample to be tested labeled as **Sample** and **Sample Background Control**. Add identical 2-10 µl of ultra-concentrate into each of these wells. For **Positive Control:** Dilute the Phytase Positive Control 4 fold with Phytase Assay Buffer prior to the assay. Add 6-10 µl of diluted Phytase Positive Control into two parallel well(s) labeled as **Positive Control** and **Enzyme Control** respectively. Adjust the volume of Positive Control and Sample wells to 20 µl/well with Phytase Assay Buffer. Adjust the volume of Sample Background Control(s) and Enzyme Control(s) wells to 50 µl/well with Phytase Assay Buffer.

Note:

a) For Unknown Samples, we suggest testing several dilutions of the sample to ensure that the readings are within the Standard Curve range.

2. Standard Curve Preparation: Mix 10 µl Glucose Standard with 990 µl Phytase Assay Buffer to prepare 1 mM Glucose Standard. Mix 10 µl of 1 mM Glucose Standard with 40 µl Phytase Assay Buffer to prepare 0.2 mM Glucose Standard solution. Add 0, 2, 4, 6, 8, 10 µl of 0.2 mM Glucose Standard solution into a series of wells to generate 0, 0.4, 0.8, 1.2, 1.6, and 2 nmol/well of Glucose Standard respectively. Adjust the volume to 20 µl/well with Phytase Assay Buffer.

3. Phytase Substrate Addition: Add 30 µl Phytase Substrate to wells containing **Positive Control**, **Sample(s)**, and **Standards**. Mix well.

4. Substrate Hydrolysis: Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl of Reaction Mix as indicated below:

	Reaction Mix	Standard Mix
Phytase Assay Buffer	43.5 µl	47.5 µl
Glucose Developer	2 µl	2 µl
Phosphate Developer A	2 µl	--
Phosphate Developer B	2 µl	--
Glucose Probe	0.5 µl	0.5 µl

Mix well and add 50 µl of Reaction Mix to each well containing **Positive Control**, **Sample(s)**, **Enzyme Control(s)**, and **Sample Background Control(s)**, mix well. Add 50 µl of Standard Mix to **Standard** wells and mix well. The total volume of each well including **Positive Control**, **Sample(s)**, **Enzyme Control**, **Sample Background Control**, and **Standards** should be **100 µl**.

Notes:

a) Have the microplate reader ready at Ex/Em 535/587 nm in kinetic mode at 37°C set to record fluorescence every 30 sec.

b) Prepare Reaction Mix immediately, before adding to the wells.

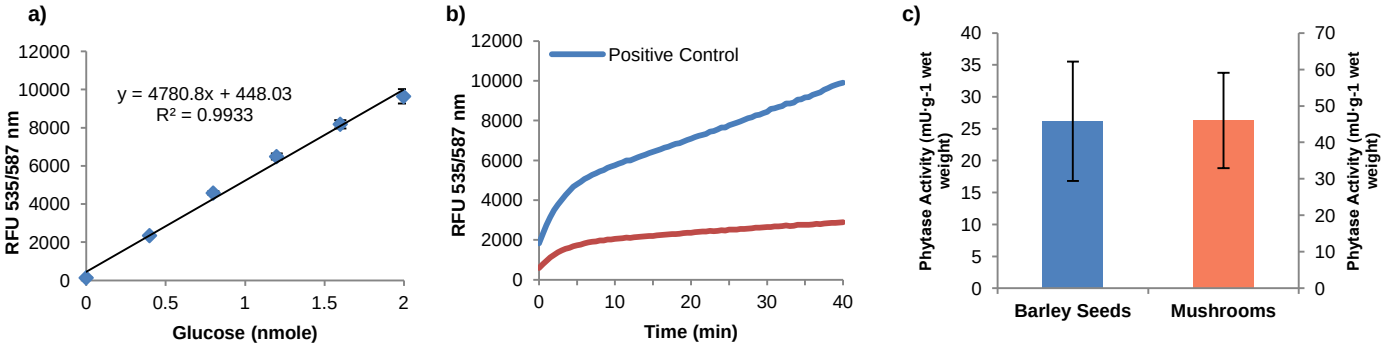
5. Measurement: Measure the fluorescence intensity of all wells at 37°C for 40 min in kinetic mode at Ex/Em = 535/587 nm. Standard Curve may be read in either kinetic or end point mode (after 40 min).

6. Calculation: Subtract the 0 Standard reading from all Standard readings and Sample Background Control reading from Sample readings respectively. Plot the Glucose Standard Curve. Choose any two time points (t_1 & t_2) within the linear portion of the curve (after the initial 10 min) for each Sample type and obtain the corresponding fluorescence values (RFU_1 and RFU_2). Apply the corrected Sample readings to the Glucose Standard Curve to get **B** nmole of glucose generated during the reaction time ($\Delta t = t_2 - t_1$).

$$\textbf{Sample Phytase Activity} = \frac{\mathbf{B \times D}}{\mathbf{\Delta t \times M \times V}} = \mathbf{nmole/min/mg} = \mathbf{mU/mg}$$

Where: **B** = Amount of Glucose produced, calculated from the Standard Curve (in nmole)
Δt = $t_2 - t_1$ (in min)
V = Sample used (ml)
M = Initial Sample concentration (mg/ml)
D = Sample dilution factor (D = 1, for undiluted samples)

Unit Definition: One unit of Phytase activity is the amount of enzyme that generates 1.0 µmol of glucose per min, at pH 5.5 at 37°C.



Figures: (a). Glucose Standard Curve, results from multiple experiments. (b). Measurement of Pytase activity (5 µg). (c). Phytase activity in barley seeds (400 µg) and mushrooms (200 µg). All assays were performed following the kit protocol.

VIII. Related Products:

Maltose Phosphorylase Activity Kit (Fluorometric) (K353)
Phosphate Assay Kit (Fluorometric) (K420)
Phosphate Colorimetric Assay Kit (K410)
Glucose Colorimetric/Fluorometric Assay Kit (K606)
10 kDa Spin Columns (1997)
Glycerol (50%), Sterile Solution (B1012)

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