ab284570 – Phytase Activity Assay Kit (Fluorometric)

For the monitoring of phytase activity.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284570

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, use kit within 2 months.

Materials Supplied

Item	Quantity	Storage Condition
Glucose Developer	1 vial	-20°C
Glucose Probe	200 µL	-20°C
Glucose Standard	100 µL	-20°C
Phosphate Developer A	1 vial	-20°C
Phosphate Developer B	50 µL	-20°C
Phytase Assay Buffer	25 mL	-20°C
Phytase Positive Control	50 µL	-20°C
Phytase Substrate	2 x 1.5 mL	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Multi-well spectrophotometer (plate reader)
- 96-Well Black Plates
- Dounce Tissue Homogenizer
- 10 kDa Spin Column
- Glycerol (50%), Sterile Solution

Reagent Preparation

- Briefly centrifuge all small vials before opening

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.

Assay Protocol

- 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. 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.
- 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.

Δ Note: *For Unknown Samples, we suggest testing several dilutions of the sample to ensure that the readings are within the Standard Curve range.*

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

Reaction Mix:

1. Phytase Substrate Addition: Add 30 µL Phytase Substrate to wells containing Positive Control, Sample(s), and Standards. Mix well.
2. 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

3. 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.
4. 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.

Δ Note: *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.*

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).

Calculation:

1. Subtract the 0 Standard reading from all Standard readings and Sample Background Control reading from Sample readings respectively.
2. Plot the Glucose Standard Curve. Choose any two time points (t1 & t2) within the linear portion of the curve (after the initial 10 min) for each Sample type and obtain the corresponding fluorescence values (RFU1 and RFU2).
3. Apply the corrected Sample readings to the Glucose Standard Curve to get B nmole of glucose generated during the reaction time (Δt = t2 - t1).

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

Where:

- B = Amount of Glucose produced, calculated from the Standard Curve (in nmole)
- Δt = t2 - t1 (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

Technical Support

For all technical or commercial enquiries please go to:

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