Phytase Assay Kit

Catalog No.: abx298913

Size: 100 Assays

Storage: Store the Standard and Positive Control at -20°C and all other kit components at 4°C.

Application: For quantitative detection of Phytase activity in tissue homogenates, cell lysates, cell culture supernatants, and other biological fluids.

Detection Range: 0.01 mmol/L – 1 mmol/L

Introduction: Phytase is an enzyme that catalyzes the hydrolysis of phytic acid (phytate). Phytic acid is a form of indigestible phosphorus found in plant-based foods such as cereals, wheats, and other grains. Hydrolysis by phytase allows animals to convert phytic acid into a usable form of phosphorus. Phytases are also found in plants, fungi, and bacteria.

Phytase decomposes the substrate provided in this kit to release phosphate ions, which react with the dye reagent. The concentration of the reaction product is directly proportional to the enzyme activity, which can be measured by measuring the absorbance at 660 nm.

Kit components

1. 96 well microplate
2. Assay Buffer: 4 × 30 ml
3. Reaction Buffer: 8 ml
4. Dye Reagent 1: 1 vial
5. Dye Reagent 2: 1 vial
6. Dye Reagent 3: 10 ml
7. Standard (1 mmol/L): 1 ml
8. Substrate: 1 vial
9. Positive Control: 1 vial
10. Plate Sealer: 3

Materials Required But Not Provided

1. Microplate reader (660 nm)
2. Convection oven
3. Microcentrifuge tubes
4. High-precision pipette and sterile pipette tips
5. Distilled water
6. Mortar
7. Centrifuge and centrifuge tubes
8. Timer
9. Ice
10. Sonicator

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Substrate Solution**
  Add 8 ml of Reaction Buffer into the Substrate vial and mix thoroughly to prepare the Substrate Solution. Ensure that the Substrate has completely dissolved prior to use.

- **Working Dye Reagent Solution**
  Add 5 ml of Dye Reagent 3 into the Dye Reagent 1 vial and mix gently. Add 1 ml of Dye Reagent 3 into the Dye Reagent 2 vial and mix gently. Ensure that the solutions are completely dissolved. Transfer the contents of the Dye Reagent 2 vial into the Dye Reagent 3 vial and mix thoroughly. Then, transfer the contents of the Dye Reagent 1 vial into the Dye Reagent 3 vial and mix thoroughly. The resulting mixture is Working Dye Reagent Solution, which can be stored at 4°C for up to 3 days.

  Note: The Working Dye Reagent Solution should be a yellow color. A blue solution indicates contamination. A colorless solution indicates incorrect mixing. It is recommended to prepare the Working Dye Reagent Solution just before carrying out the assay.

  All waste should be disposed appropriately. Please note that due to the inorganic phosphate reaction products, you may need to follow special waste disposal procedures. Please check local disposal regulations.

- **Positive Control Solution**
  Add 1 ml of distilled water to the Positive Control vial and mix thoroughly. Ensure that the Positive Control has completely dissolved. Take 0.1 ml of this solution and add 0.9 ml of distilled water to prepare the Positive Control Solution. The Positive Control Solution should be used immediately or stored at -20°C.

2. Sample

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant, and add 1 ml of Assay Buffer for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 8000 × g at 4°C for 20 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer on ice. Centrifuge at 8000 × g at 4°C for 20 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

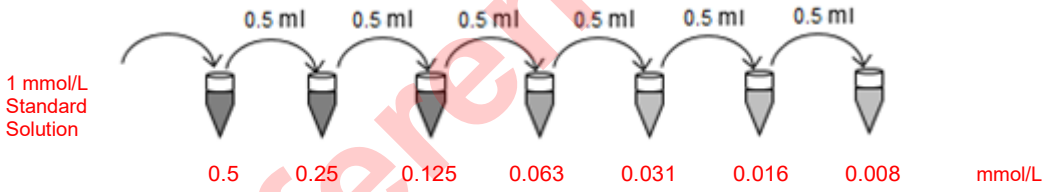
- **Liquid samples**
  Liquid samples can be used directly.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

If the expected activity is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured activity within the detection range of the kit.

1. Label 8 tubes with 0.5 mmol/L, 0.25 mmol/L, 0.125 mmol/L, 0.063 mmol/L, 0.031 mmol/L, 0.016 mmol/L, and 0.008 mmol/L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 1 mmol/L standard solution to the 1st tube (0.5 mmol/L) and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set the sample, standard, blank and positive control wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 80 µl of Substrate Solution to the sample, blank and positive control wells. Leave the standard wells empty with no liquid.
4. Add 20 µl of sample to the sample wells.
5. Add 20 µl of distilled water to the blank wells.
6. Add 20 µl of Positive Control Solution to the positive control wells.
7. Add 100 µl of prepared standard solutions to the standard wells.
8. Tap the plate gently to mix. Cover the plate with a plate sealer and incubate in a convection oven at 55°C for 10 minutes.
9. Add 100 µl of Working Dye Reagent Solution to all wells.
10. Tap the plate gently to mix. Read and record absorbance at 660 nm.

C. Calculations

One unit of Phytase activity is defined as the amount of enzyme required to produce 1 µmol of PO_4^{3-} per minute.

Phytase activity per mg of protein:

$$\textbf{Phytase}\ (\mathbf{U/mg}) = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}}}{\mathbf{V_{Sample}} \times \mathbf{C_{Protein}} \times \mathbf{T}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \frac{\mathbf{0.5}}{\mathbf{C_{Protein}}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

Phytase activity per g of sample:

$$\textbf{Phytase}\ (\mathbf{U/g}) = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{V_{Sample} \times W \times T}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{0.5}}{\mathbf{W}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

Phytase activity per 10^4 cells or bacteria:

$$\textbf{Phytase}\ (\mathbf{U/10^4\ cells}) = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{V_{Sample} \times N \times T}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{0.5}}{\mathbf{N}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

Phytase activity per ml of sample:

$$\textbf{Phytase}\ (\mathbf{U/ml}) = \frac{\mathbf{C_{Standard} \times V_{Standard}}}{\mathbf{V_{Sample} \times T}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \mathbf{0.5} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

where:

$\mathbf{C_{Protein}}$	Concentration of protein (in mg/ml)
$\mathbf{C_{Standard}}$	Concentration of highest standard (1 mmol/L = 1 µmol/ml)
T	Reaction time (10 minutes)
W	Weight of the sample (in g)
N	Number of cells or bacteria (× 10^4)
$\mathbf{V_{Assay}}$	Volume of assay buffer (1 ml)
$\mathbf{V_{Sample}}$	Volume of sample (0.02 ml)
$\mathbf{V_{Standard}}$	Volume of standard (0.1 ml)