Phytic Acid Assay Kit

Catalog No.: abx298933

Size: 100 Assays

Storage: Store the Enzyme at -20°C and all other components at 4°C.

Application: For quantitative detection of Phytic Acid concentration in tissue homogenates, cell lysates, cell culture supernatants, and other biological fluids.

Detection Range: 0.04 mmol/L – 4 mmol/L

Introduction: Phytic acid, also known as inositol hexakisphosphate (IP6) or inositol polyphosphate, is a six-fold dihydrogenphosphate ester of inositol. It functions as the principal storage form of phosphorus in many plant tissues. At physiological pH, the phosphates are partially ionized, resulting in the phytate anion.

Abbexa's Phytic Acid Assay Kit is designed to directly measure Phytic Acid concentrations in a variety of samples. In this assay, phytase decomposes phytic acid, releasing a phosphate ion which reacts with the dye reagent. The concentration of the reaction product is directly proportional to the Phytic Acid concentration in the sample and can be calculated by measuring the absorbance at 660 nm.

Kit components

1. 96 well microplate
2. Reaction Buffer: 8 ml
3. Enzyme: 1 vial
4. Dye Reagent 1: 1 vial
5. Dye Reagent 2: 1 vial
6. Dye Reagent 3: 10 ml
7. Standard: 1 vial
8. Plate sealer: 3

Materials Required But Not Provided

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

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Enzyme Solution**
  Add 1.1 ml of distilled water into the Enzyme vial and mix thoroughly to prepare the Enzyme Solution. Centrifuge at 4000 × g for 5 minutes, then use immediately or aliquot and store at -20°C. Ensure that the Enzyme has completely dissolved prior to use.

- **Standard Solution**
  Add 1.25 ml of distilled water into the Standard vial and mix thoroughly to prepare the Standard Solution (concentration 4 mmol/L). Ensure that the Standard 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 reagents 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. The resulting mixture is the Working Dye Reagent Solution, which can be stored at 4°C for up to 3 days.

  Note: The Working Dye 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 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.

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 distilled water 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 pre-cooled tube, then analyze immediately.

- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of distilled water on ice. Centrifuge at 8000 × g at 4°C for 20 minutes. Transfer the supernatant to a new pre-cooled tube, then 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 concentration is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured concentration within the detection range of the kit.

1. Label 7 tubes with 2 mmol/L, 1 mmol/L, 0.5 mmol/L, 0.25 mmol/L, 0.125 mmol/L, 0.063 mmol/L, and 0.031 mmol/L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 4 mmol/L Standard Solution to the 1st tube 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 and blank wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 10 µl of sample to the sample wells.
4. Add 10 µl of prepared standards to the standard wells.
5. Add 10 µl of distilled water to the blank wells.
6. Add 80 µl of Reaction Buffer to all wells.
7. Add 10 µl of Enzyme Solution to all wells.
8. Tap the plate gently to mix. Cover the plate with a plate sealer and incubate 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

Phytic Acid concentration per mg of protein:

$$\textbf{Phytic Acid } (\mu mol/mg) = \frac{C_{Standard} \times V_{Standard}}{V_{Sample} \times C_{Protein}} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} = \frac{4}{C_{Protein}} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}}$$

Phytic Acid concentration per g of sample:

$$\textbf{Phytic Acid } (\mu mol/g) = \frac{C_{Standard} \times V_{Standard} \times V_{Assay}}{V_{Sample} \times W} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} = \frac{4}{W} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}}$$

Phytic Acid concentration per 10^4 cells or bacteria:

$$\textbf{Phytic Acid } (\mu mol/10^4 \text{ cells}) = \frac{C_{Standard} \times V_{Standard} \times V_{Assay}}{V_{Sample} \times N} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} = \frac{4}{N} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}}$$

Phytic Acid concentration per ml of sample:

$$\textbf{Phytic Acid } (\mu\text{mol/ml}) = \frac{C_{Standard} \times V_{Standard}}{V_{Sample}} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} = 4 \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}}$$

where:

$C_{Protein}$	Concentration of protein (in mg/ml)
$C_{Standard}$	Concentration of highest standard (4 mmol/L = 4 µmol/ml)
W	Weight of the sample (in g)
N	Number of cells or bacteria (× 10^4)
V_{Assay}	Volume of distilled water (1 ml)
V_{Sample}	Volume of sample (0.01 ml)
$V_{Standard}$	Volume of standard (0.01 ml)