Phytase Activity Assay kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer

Catalog Number: NA0399

Size: 100T/48S

Components:

Extraction Reagent: Liquid 30 mL×1. Storage at 2-8℃;

Buffer: Liquid 30 mL×1. Storage at 2-8℃;

Reagent I: Powder×1. Storage at 2-8℃; Mix reagent 1 and buffer solution before use (you can draw buffer into reagent 1 bottle for repeated washing). Prepare when the solution will be used. Unused reagents can be stored for 4 weeks at 2-8℃;

Reagent II: Powder×1. Storage at 2-8℃, Before use, add 6.3mL of distilled water to fully dissolve, and then put the pipette tip under the liquid surface and slowly add 1.7mL of concentrated sulfuric acid, Unused reagents can be stored for 4 weeks at 2-8℃;

Reagent III: Powder×1. Storage at 2-8℃, add 40mL of distilled water to fully dissolve, Unused reagents can be stored for 4 weeks at 2-8℃;

Working Solution: Before use, mix reagent II and reagent III at the ratio of 1mL:5mL (about 10 tubes) according to the measured quantity. Unused reagents can be stored for 3 days at 2-8℃.

Standard Solution: Liquid 1 mL×1. Storage at 2-8℃. Inorganic phosphorus standard solution with a concentration of 5 μmol/mL.

Product Description

Phytase (Phytase), is a binding enzyme of protein and sugar. Phytase can decompose phytic acid to produce inorganic phosphorus and inositol, which greatly improves the utilization rate of nutrients by organisms. Phytase widely exists in plants, animal tissues and microorganisms. Now, microorganisms are used to synthesize phytase for production and application. Phytase has extensive research value in the fields of food production and animal husbandry.

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

Reagents and Equipment Required but Not Provided.

Spectrophotometer, Table Centrifuge, Water-bath/Constant Temperature Incubator, 1 mL Glass Cuvette, Mortar/Homogenate/Cell ultrasonic Crusher, Ultrasonic Dissolver, Gyrotron Oscillator, Ice and Distilled Water, Concentrated sulfuric acid.

Procedure

I. Sample processing

1. Tissue: add the extract solution according to the ratio of mass(g): volume of extract solution(mL): 1:5~10 (it is recommended to weigh about 0.1g and add 1 mL of extract solution, homogenize in ice bath and centrifuge at 4℃, 4000g for 10 min, and take supernatant on ice before testing.

2. Cells: according to the number of cells (10^4): the volume of extract solution (mL) is 500-1000:1 (it is recommended to add 1 mL extract solution to 5 million cells), ice bath ultrasonic wave is used to crush cells (power 200W, ultrasonic 3s, interval 7s, total time 3 min), and take supernatant on ice before testing.

3. Serum: direct determination. If the solution is turbid, centrifuge and take the supernatant for measurement.

II. Determination procedure:

1. Preheat spectrophotometer for more than 30 minutes, adjust the wavelength to 700 nm, set the counter to zero with distilled water.

2. Dilution of standard solution: Dilute 5μmol/mL inorganic phosphorus standard solution with distilled water to 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.0156 μmol/mL for use.

3. Sample determination (adding the following reagents to the EP tube):

Reagent (μL)	Test tube(A_T)	Contrast tube(A_C)	Standard tube(A_S)	Blank tube(A_B)
Sample	200	200	-	-
Standard solution	-	-	200	-
Stay in 37℃ water bath for 5 minutes.			-	-
Reagent I	480	-	-	-
Mix thoroughly. Stay in 37℃ water bath for 30 minutes, then stay in 100℃ water bath for 10 minutes.			-	-
Reagent I	-	480	-	-
Distilled water	-	-	480	680
Working solution	600	600	600	600
Mix thoroughly. Stay in room temperature for 10 minutes. Centrifuge at 8000g， room temperature for 10 minutes. Take 1 mL of supernatant in 1 mL glass cuvette. Measure the absorbance at 700 nm. and record it as A_T、A_C、A_S and A_B respectively. $\Delta A_T=A_T-A_C$, $\Delta A_S=A_S-A_B$, The standard curve and blank tube only need to be measured 1-2 times. Each Test tube needs to be provided with a control tube.				

III. Calculation

1. Make standard curve:

Get the standard curve according to concentration of standard solution(x,nmol/mL) and absorbance (y, ΔAs). According to the standard curve, take ΔA(y) into the formula to get the concentration of sample (x,μmol/mL).

2. Calculation:

(1) Calculate by protein concentration:

Unit definition: Under the condition of 37℃, pH 5.5, one unit of enzyme activity is defined as the amount of enzyme catalyzes the release of 1 μmol of inorganic phosphorus in the reaction system per minute every milligram tissue protein.

Phytase Activity (U/mg prot)＝$x \times V_E \div (V_E \times Cpr) \div T = x \div Cpr \div 30$.

(2) Calculate by sample weight:

Unit definition: Under the condition of 37℃, pH 5.5, one unit of enzyme activity is defined as the amount of enzyme catalyzes the release of 1 μmol of inorganic phosphorus in the reaction system per minute every gram tissue.

Phytase Activity (U/g weight)＝$x \times V_E \div W \div T = x \div W \div 30$.

(3) Calculate by number of bacteria or Cultured Cells:

Unit definition: Under the condition of 37℃, pH 5.5, one unit of enzyme activity is defined as the amount of enzyme catalyzes the release of 1 μmol of inorganic phosphorus in the reaction system per minute every 10^4 cells.

Phytase Activity (U/10^4 cell)＝$x \times V_E \div N \div T = x \div N \div 30$.

(4) Calculated by liquid volume

Unit definition: Under the condition of 37℃, pH 5.5, one unit of enzyme activity is defined as the amount of enzyme catalyzes the release of 1 μmol of inorganic phosphorus in the reaction system per minute every milliliter liquid.

Phytase Activity (U/mL)＝$x \times V_S \div V_S \div T = x \div 30$

V_S: Sample volume, 0.2 mL;

V_E: Extraction Reagent volume, 1 mL;

Cpr: Sample protein concentration, mg/mL;

W: Weight of the sample, g;

N: Number of cells (Unit: 10^4);

T: Reaction time, 30 min.

Note

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

2. If the measured absorbance value is too low or close to blank, appropriately extend the reaction time of the 37°C water bath in the second step or increase the sample size, and then re-measure. If the A determination is greater than 1.5 or the ΔA exceeds the detection range, it is recommended that the sample be properly diluted with distilled water for determination. Note that the calculation formula is modified synchronously.

3. The final liquid should be measured within 40 minutes.

Experimental example:

Take 0.15g spinach seeds, add 1 mL of extract solution, homogenize in ice bath and centrifuge at 4℃, 4000g for 10 min, and take supernatant on ice before testing, then operate according to the determination steps, and calculate: $\Delta A_T = A_T - A_C = 1.327 - 1.054 = 0.273$, Substitute ΔA into the standard curve formula y = 0.4892x + 0.0313, and get x= 0.49, and calculate the enzyme activity according to the sample mass:

Phytase Activity (U/g weight) =0.1098 U/g weight.

References：

[1] Senna R , Simonin V , Silva-Neto M A C , et al. Induction of acid phosphatase activity during germination of maize (Zea mays) seeds[J]. Plant Physiology & Biochemistry, 2006, 44(7-9):467-473.

[2] Iqbal T H , Lewis K O , Cooper B T . Phytase activity in the human and rat small intestine[J]. Gut, 1994, 35(9):1233-1236.

[3] Azeke M A , Egielewa S J , Ihimire E . Effect of germination on the phytase activity, phytate and total phosphorus contents of rice (Oryza sativa), maize (Zea mays), millet (Panicum miliaceum), sorghum (Sorghum bicolor) and wheat (Triticum aestivum)[J]. Journal of Food Science&Technology, 2011.

Related Products：

NA0378/NA0440 Basic xylanase (BAX) Activity Assay Kit

NA0688/NA0447 Cellulase (CL) Assay Kit

NA0838/NA0596 β-1,3-glucanase(β-1,3-GA) Activity Assay Kit

NA0820/NA0578 α-amylase Assay Kit