

## Soil Phytase Activity Assay Kit

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

**Operation Equipment:** Spectrophotometer/Microplate reader

**Catalog Number:** NA0134

**Size:** 100T/48S

### Components:

**Reagent I:** Liquid 3 mL×1, storage at 2-8°C. Toluene (self-provided).

**Reagent II A:** Powder×1, storage at 2-8°C.

**Reagent II B:** Liquid 60 mL×1, storage at 2-8°C. Pour all reagent II B into reagent II A before use, fully shake and dissolve it, the unused reagent can be stored at 2-8°C for 4 weeks.

**Reagent III:** Powder×1, storage at 2-8°C. Before use, add 3 mL distilled water to fully dissolve it. Then slowly add 0.81mL concentrated sulfuric acid under the liquid level. The unused reagent can be stored at 2-8°C for 4 months.

**Reagent IV:** Powder×1, storage at 2-8°C. Before use, add 13 mL distilled water to fully dissolve it. Then slowly add 20μL concentrated sulfuric acid. The unused reagent can be stored at 2-8°C for 4 months.

**Working Solution:** Before use, mix the proportion of Reagent III: Reagent IV=1mL: 5mL (about 60T, which can be adjusted proportionally according to the sample size), the unused reagent can be stored at 2-8°C for 3 days.

**Standard:** Liquid 1 mL×1, storage at 2-8°C. 5 μmol/mL inorganic phosphorus standard solution.

### Product Description:

Phytase, also known as inositol hexaphosphatase, is an enzyme that combines protein and sugar. Phytase can decompose phytic acid to produce inorganic phosphorus and inositol, greatly improving the utilization of nutrients by organisms. Soil phytase mainly comes from soil microorganisms and plays an important role in the phosphorus cycle. Soil phytase has a strong application prospect in the field of soil improvement and sustainable agricultural development.

Under certain environmental conditions, soil phytase can decompose sodium phytate (twelve sodium inositol hexaphosphate) to produce inorganic phosphorus and inositol derivatives. Under acidic conditions, inorganic phosphorus reacts with ammonium molybdate chromogenic agent to produce blue molybdenum blue substance, which has a characteristic absorption peak at 700nm. The activity of soil phytase can be calculated by measuring the content of inorganic phosphorus.

### Reagents and Equipments Required but Not Provided:

Spectrophotometer/Microplate Reader, Centrifuge, Water-bath/Constant Temperature Incubator, Transferpette, Micro Glass Cuvette/96 Well Flat-bottom Plate, Mortar, Oscillator, Toluene, Concentrated Sulfuric acid, 30-50 Mesh Sieve, Ice and Distilled Water.

## Procedure:

### I. Sample preparation:

Fresh soil samples are naturally air-dried or oven to dry at 37°C, then sieved by 30 ~ 50 mesh sieve.

### II. Determination procedure:

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 700 nm, set zero with distilled water.
2. Dilute the 5  $\mu\text{mol/mL}$  standard solution to 1.25, 0.625, 0.3125, 0.15625, 0.078125, 0.039, 0.02, 0.01, 0.005 $\mu\text{mol/mL}$  standard with distilled water.
3. Add reagents with the following list (add the following reagents into the EP tube in turn):

Reagent	Test Tube (T)	Contrast Tube (C)	Standard tube (S)	Blank tube (B)
Soil Sample (g)	0.03	0.03	-	-
Reagent I ( $\mu\text{L}$ )	20	20	-	-
Thoroughly shake and mix, placed at room temperature for 15min.			-	-
Reagent II ( $\mu\text{L}$ )	500	-	-	-
Evenly mixed, react at 37 °C (water-bath/Constant temperature incubator) for 24h, place in boiling water for 10min.			-	-
Reagent II ( $\mu\text{L}$ )	-	500	-	-
Centrifuge at 10000g and 25 °C for 5min, take the supernatant.			-	-
Standard Solution( $\mu\text{L}$ )	-	-	100	-
Distilled Water( $\mu\text{L}$ )	-	-	-	100
Supernatant( $\mu\text{L}$ )	100	100	-	-
Working Solution( $\mu\text{L}$ )	100	100	100	100

Thoroughly shake and mix, placed at room temperature for 15min, measure the light absorption value at 700nm, record it as  $A_T$ ,  $A_C$ ,  $A_S$  and  $A_B$ ,  $\Delta A = A_T - A_C$ ,  $\Delta A_S = A_S - A_B$ . Note: The standard curve and blank tube only need to be measured 1-2 times. Each test tube shall be equipped with a contrast tube.

### III. Soil Phytase activity calculation:

1. Make standard curve:

Get the standard curve according to concentration of standard solution(x,  $\mu\text{mol/mL}$ ) and absorbance (y,  $\Delta A_S$ ). According to the standard curve, take  $\Delta A(y)$  into the formula to get the concentration of sample (x,  $\mu\text{mol/mL}$ ).

2. Calculation of soil phytase activity

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the release of 1  $\mu\text{mol}$  of inorganic phosphorus in the reaction system per day at 37°C every gram of soil sample.

$$\text{Soil Phytase Activity (U/g)} = x \times V_{rv} \div W \div T = 0.52x \div W$$

$V_{rv}$ : Total volume in catalyze system, 0.52mL;

W: Soil sample weight, g;

T: Reaction time, 24h=1d;

**Notes:**

1. The temperature of 100 °C boiling water bath is relatively high, so it is recommended to wrap the centrifugal tube with sealing film or use the centrifugal tube with spiral cover.
2. If the  $\Delta A < 0.01$ . After appropriately extending the reaction time at 37 °C in the first step or increasing the sample size, re measure. If  $\Delta A > 1.2$ , it is recommended to dilute the supernatant with distilled water properly before measurement. Note to multiply the result by the dilution factor
3. Please complete the measurement within 30min, and try to ensure the consistency of the measurement time of all samples.

**Experimental instances:**

1. Take 0.03g clover soil, operated according to the determination steps, Measured with 1mL glass cuvette,  $A_T = 0.359$ ,  $A_c = 0.28$ ,  $\Delta A = 0.079$ , the standard equation is:  $y = 1.1104 x + 0.0077$ ,  $x = 0.0642$ , calculate the soil phytase activity:

Soil Phytase Activity (U/g)= 1.113U/g.

2. Take 0.03g mushroom soil, operated according to the determination steps, Measured with 1mL glass cuvette,  $A_T = 0.442$ ,  $A_c = 0.32$ ,  $\Delta A = 0.122$ , the standard equation is:  $y = 1.1104 x + 0.0077$ ,  $x = 0.1029$ , calculate the soil phytase activity:

Soil Phytase Activity (U/g)= 1.784U/g.

**References:**

[1] Berry D F, Shang C, Zelazny L W. Measurement of phytase activity in soil using a chromophoric tethered phytic acid probe[J]. Soil Biology & Biochemistry, 2009, 41(2):192-200.

[2] Marshall, Arebojie, Azeke, et al. 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, 48(6):724-729.

**Related Products:**

- NA0860/NA0617 Soil acid phosphatase(S-ACP) Activity Assay Kit
- NA0830/NA0588 Soil Neutral Phosphatase (S-NP) Assay Kit
- NA0846/NA0604 Soil Alkaline Phosphatase(S-AKP/ALP) Activity Assay Kit
- NA0847/NA0605 Soil Neutral Protease Activity Assay Kit
- NA0836/NA0594 Soil Dehydrogenase Assay Kit

