Soil Neutral Phosphatase Assay Kit

Cat#: orb1499878 (manual)

Size: 100T/96S

Microassay

Product composition and storage conditions:

No.	Specifications	Storage Conditions
orb1499878 - A	50 ml ×1	Store at room temperature;
orb1499878 - B	Powder ×1	Store at 4°C, dissolve in 100 mL distilled water before use;
orb1499878 - C	5ml ×1	Store at 4°C;
orb1499878 - D	Powder ×1	Store at 4 ° C and protected from light. Add 576 μL of anhydrous ethanol (self-prepared) and 24 μL of distilled water to fully dissolve before use (Cannot be used after browning);
orb1499878 – Standard (0.5μmol/mL)	1ml ×1	Store at 4°C (Phenol standard solution).

※Before the formal measurement, be sure to take 2-3 samples with large expected differences for predetermination.

Introduction:

Significance: Soil phosphatase is the enzyme that catalyzes soil organophosphorus mineralization. Its activity directly affects the decomposition and bioavailability of organophosphorus in soil. It is an index to evaluate the direction and intensity of soil phosphorus biotransformation. The activity of phosphatase was significantly affected by the content of soil carbon, nitrogen, available phosphorus and pH. According to the optimal pH range, soil phosphatase was divided into three types: Neutral, acidic and alkaline.

Principle: In a neutral environment, S-NP catalyzes the hydrolysis of benzophenyldisodium phosphate to phenol and disodium hydrogen phosphate, and NP activity can be calculated by measuring the amount of phenol produced.

Own supplies:

Visible spectrophotometer/microplate reader, microquartz cuvette/96-well plate, centrifuge, 37℃ incubator, analytical balance, adjustable pipette, ice, distilled water, ethanol and toluene.

Catalytic reaction:

Weigh about 0.1 g air-dried and mixed soil, add 50μL toluene, and shake gently for 15 min; Add 400μL orb1499878 -A and shake well, place in 37℃ incubator and catalyze the reaction for 24 h; After that, immediately add 1mL orb1499878 -B and mix well to stop the enzyme-catalyzed reaction. Centrifuge with 8000 g at 25℃ for 10 min, and put the supernatant on ice for test.

Measurement steps:

1. Preheat the visible spectrophotometer/microplate reader for at least 30 minutes, adjust the wavelength to 660 nm, set zero with distilled water.

2. Add the following reagents in sequence to the EP tube:

Reagent name	Blank tube (ul)	Standard tube (ul)	Measuring tube (ul)
Distilled water	10		
Standard		10	
Supernatant			10
orb1499878 - C	20	20	20
orb1499878 - D	4	4	4
Mix well and add distilled water after color development.			
Distilled water	166	166	166
After mixing, stand for 30 min at RT, measure absorbance at 660 nm, and record as A blank, A standard and A measuring. Note: The blank and standard tubes need only be determined once.			

S-ACP activity calculation:

Definition of active unit: 1nmol phenol released per day per gram of soil in 37℃ is one enzyme active unit.

S-ACP (nmol/d/g) = [C standard × (A measuring -A blank) ÷ (A standard -A blank)] ×V total ÷W÷T =

725 × (A measuring - A blank) ÷ (A standard -A blank) ÷W

Note: C Standard: 0.5 μmol/mL; V total: Total volume of catalytic system, 1.45mL; W: Soil sample weight, g; T: Catalytic reaction time, 24 h=1 d.

Precautions:

1. The reagent has certain harm to human body, please wear experimental clothes and gloves.

2. Linearity range is 0.03125 μmol/mL-3μmol/mL.