Soil acid phosphatase Assay Kit

Cat#: orb1499892 (manual)

Size: 100T/96S

Microassay

Product composition and storage conditions:

No.	Specifications	Storage Conditions
orb1499892 - A	42ml ×1	Store at 4 ° C and protected from light;
orb1499892 - B	Powder ×1	Store at 4°C, dissolve in 100 mL distilled water before use;
orb1499892 - C	5ml ×1	Store at 4°C;
orb1499892 - 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);
orb1499892 – Standard (0.1umol/ml)	1ml ×1	Store at 4°C.

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

Introduction:

Significance: Soil acid phosphatase (S-ACP) is a kind of enzyme that catalyzes the mineralization of organic phosphorus in soil. Its activity directly affects the decomposition and bioavailability of organic phosphorus in soil. It is an index to evaluate the direction and intensity of soil phosphorus biotransformation. Soil phosphatase is significantly affected by soil carbon, nitrogen content, available phosphorus content and pH. According to the optimal pH range, it can be divided into three types: acidic, neutral and alkaline.

Principle: In acidic environment, S-ACP catalyzes the hydrolysis of disodium phosphate to form phenol and disodium hydrogen phosphate. The activity of S-ACP can be calculated by determining the amount of phenol produced.

Own supplies:

Visible spectrophotometer/microplate reader, microquartz cuvette/96-well plate, centrifuge, 37℃ incubator, analytical balance, adjustable pipette, 30-50 mesh sieve, ice, distilled water, ethanol and toluene.

Crude enzyme extract:

1. Fresh soil sample is air-dried naturally or at 37℃ incubator, passing 30~50 mesh sieve.

2. Weigh about 0.1 g air-dried and mixed soil, add 50μL toluene, and shake gently for 15 min; Add 0.4 mL orb1499892 -A and shake well, place in 37℃ incubator and catalyze the reaction for 24 h; After that, immediately add 1mL orb1499892 -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
orb1499892 - C	20	20	20
orb1499892 - D	4	4	4
Mix well and add distilled water after color development.			
Distilled water	166	166	166
After mixing, leave room temperature to stand for 30 min, 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 1-2 times.			

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 (U/g) = [C standard × (A measuring -A blank) ÷ (A standard -A blank)] ×V total × 1000 ÷W÷T =

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

Note: C Standard: 0.1 μmol/mL; V total: Total volume of catalytic system, 1.45mL; W: Soil sample weight, g; T: Catalytic reaction time, 24 h=1 d, 1000: Unit conversion factor, 1μmol=1000nmol.