Soil Alkaline Phosphatase Assay Kit

Cat#: orb1499896 (manual)

Size: 100T/96S

Microassay

Product composition and storage conditions:

No.	Specifications	Storage Conditions
orb1499896 - A	42mL ×1	Store at 4°C, protected from light;
orb1499896 - B	Powder ×1	Store at 4°C; Add 100mL distilled water to fully dissolve before use;
orb1499896 - C	5mL ×1	Store at 4°C;
orb1499896 - D	Powder ×1	Store at 4°C, protected from light. Add 576 μL of absolute ethanol (self-prepared) and 24μL distilled water to fully dissolve before use (cannot be used after browning);
orb1499896 -Standard 0.5umol/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 Alkaline Phosphatase, S-AKP/ALP is a kind of enzyme that catalyzes the mineralization of soil organophosphorus compounds. 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. Soil phosphatase was significantly affected by soil carbon, nitrogen, available phosphorus and pH. It is usually divided into three types of phosphatase, basic, neutral and acidic, according to their optimum pH range. This is the soil alkaline phosphatase (S-AKP/ALP) test testing kit.

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

Own supplies:

Visible photometer/microplate reader, microglass cuvette/96-well plate, bench top centrifuge, 37℃ incubator, analytical balance, adjustable pipette, ice, distilled water, anhydrous ethanol and toluene.

Catalytic reaction:

Weigh about 0.1 g of air-dried and mixed soil, add 50μL of toluene ((self-prepared), and shake gently for 15 min; Add 0.4 mL orb1499896 -A and shake well, place in 37℃ incubator and catalyze the reaction for 24 h; After that, immediately add 1mL orb1499896 -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 660nm, set zero with distilled water.
2. Add the following reagents in sequence to the microglass cuvette/96-well plate:

Reagent name	Blank tube (ul)	Standard tube (ul)	Measuring tube (ul)
Distilled water	10		
Standard		40	
Supernatant			10
orb1499896-C	20	20	20
orb1499896-D	4	4	4
Mix well and add distilled water after color development			
Distilled water	166	166	166
After mixing, allow to stand for 30 min at 25℃, measure the absorbance at 660 nm, and record as A blank, A standard, A measuring.			

Note: Blank and standard tubes need to be determined only once.

S-ALP activity calculation formula:

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

S-AKP/ALP (nmol/d/g) = [C standard × (A measuring -A blank) ÷ (A standard -A blank)] ×V total ÷ W ÷ T× 1000 = 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; 1000: Unit conversion factor, 1μmol=1000nmol.