

Soil Alkaline Phosphatase Assay Kit

Cat#: orb1499897 (manual)

Size: 50T/48S

Visible Spectrophotometer

Product composition and storage conditions:

No.	Specifications	Storage Conditions
orb1499897 - A	21 ml ×1	Store at 4 ° C and protected from light;
orb1499897 - B	Powder ×1	Store at 4°C, dissolve in 50 mL distilled water before use;
orb1499897 - C	5ml ×1	Store at 4°C;
orb1499897 - D	Powder ×1	Store at 4 ° C and protected from light. Add 1152 µL of anhydrous ethanol (self-prepared) and 48 µL of distilled water to fully dissolve before use (Cannot be used after browning);
orb1499897 – Standard (0.5µmol/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. Usually according to their optimum pH range, it is divided into three types of phosphatase: alkaline, neutral and acidic. 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 spectrophotometer, centrifuge, 37°C incubator, analytical balance, adjustable pipette, 1 mL glass cuvette, 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 orb1499897 -A and shake well, place in 37°C incubator and catalyze the reaction for 24 h; After that, immediately add 1mL orb1499897 -B and mix well to stop the enzyme-catalyzed reaction. Centrifuge with 8000 g at 25°C for 10 min, and put the supernatant on ice for test.

Measurement steps:

1. Preheat the visible spectrophotometer 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	50		
Standard		50	
Supernatant			50
orb1499897 - C	100	100	100
orb1499897 - D	20	20	20
Mix well and add distilled water after color development.			
Distilled water	830	830	830
After mixing, stand for 30 min at 25°C, 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°C is one enzyme active unit.

$$S\text{-AKP/ALP (nmol/d/g)} = [C \text{ standard} \times (A \text{ measuring} - A \text{ blank}) \div (A \text{ standard} - A \text{ blank})] \times V \text{ total} \div W \div T \times 1000 = 725 \times (A \text{ measuring} - A \text{ blank}) \div (A \text{ standard} - A \text{ blank}) \div W$$

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.

Linearity range: 0.03125 µmol/mL -2.5 µmol/mL