

Soil aryl sulfatase (S-ASF) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Microplate reader/ Spectrophotometer

Catalog Number: AK0125

Size:100T/48S

Components:

Reagent I: 2 mL×1, Toluene (Provide for oneself). stored at 4°C .

Reagent II: 12 mL×1. Storage at 4°C .

Reagent III: Powder×2. Storage at -20°C . Before use, add 5 mL of distilled water into each bottle, fully dissolve it for standby. The reagents that cannot be used up can be packed separately and stored at -20°C to avoid repeated freezing and thawing.

Reagent IV:30 mL×1. Storage at 4°C

Standard: 1 mL×1. 5 mmol/L of p-nitrophenol solution. Dilute the standard 50 times with Reagent II to obtain 100 μmol/L standard solution before use.

Product Description

Soil aryl sulfatase come from soil microorganism, which can catalyze the transformation of soil organic sulfide into plant absorbable inorganic sulfur. It plays an important role in the biochemical cycle of sulfur and sulfur nutrition metabolism of plants, and it is an important biological index reflecting soil quality.

S-ASF can catalyzes the formation of p-nitrophenol from p-nitrobenzenesulfate, which has characteristic light absorption at 410 nm.

Reagents and Equipment Required but Not Provided.

Spectrophotometer/microplate reader, table centrifuge, water-bath, transferpettor, micro glass cuvette/96 well plate, mortar, ice, 30-50 mesh sieve (or smaller), toluene (express delivery is not allowed) and distilled water.

Procedure

I. Sample processing:

The fresh soil samples are dried naturally or in the oven at 37°C and screened with 30-50 mesh.

II. Determination steps:

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 410 nm, set zero with the distilled water.
2. Add reagents in turn according to the following table:

Reagent name	Test tube (T)	Contrast tube (C)	Standard tube(S)	Blank tube(B)
Air dried soil sample (g)	0.02	0.02	-	-
Reagent I (μL)	10	10	-	-

Shake and mix well, make the soil sample all wet, and place it at room temperature for 15 minutes.			-	-
Reagent II (μL)	100	100	-	-
Reagent III (μL)	80	-	-	-
Mix well, after reacting at water bath for 1 hour at 37°C, immediately boil for 5 minutes (cover tightly to prevent water loss), and cool in water/ice bath.			-	-
Reagent III (μL)	-	80	-	-
Centrifugate at 10000 rpm for 1 minutes at 25°C, take the supernatant.			-	-
Supernatant (μL)	100	100	-	-
Standard (μL)	-	-	100	-
Distilled water (μL)	-	-	-	100
Reagent IV (μL)	200	200	200	200

Mix well. After standing at room temperature for 2 minutes, take 200 μL into micro glass cuvette/96 well plate to measure the absorbance value. Record it as A_T , A_C , A_S and A_B respectively. Calculate $\Delta A = A_T - A_C$, $\Delta A_S = A_S - A_B$. Each test tube is provided with contrast tube.

III. Calculate activity of S-ASF

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 μmol ofp-nitrophenol per day every gram soil sample.

$$\text{S-ASF (U/g soil sample)} = \Delta A \div (\Delta A_S \div C_S) \times V_{RT} \div W \div T = 0.456 \times \Delta A \div \Delta A_S \div W$$

T: Reaction time, 1 hour=1/24 day;

V_{RT} : Total volume of reaction system: 1.9×10^{-4} L;

C_S : Concentration of standard solution, 100 μmol/L;

W: Sample quality, g.

Experimental examples:

1. Take two tubes of 0.1g clover soil and mark them as test tube and control tube respectively, and follow the measurement procedure. After determination with 96 well plate, calculate $\Delta A = A_T - A_C = 0.401 - 0.076 = 0.325$, $\Delta A_S = A_S - A_B = 0.395 - 0.047 = 0.348$. The enzyme activity is calculated according to the sample mass.

$$\text{S-ASF (U/g soil sample)} = 0.456 \times \Delta A \div \Delta A_S \div W = 21.293 \text{ U/g.}$$

2. Take two tubes of 0.1g soil sample and mark them as test tube and control tube respectively, and follow the measurement procedure. After determination with 96 well plate, calculate $\Delta A = A_T - A_C = 0.262 - 0.066 = 0.196$, $\Delta A_S = A_S - A_B = 0.395 - 0.047 = 0.348$. The enzyme activity is calculated according to the sample mass.

$$\text{S-ASF (U/g soil sample)} = 0.456 \times \Delta A \div \Delta A_S \div W = 12.841 \text{ U/g.}$$

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