Soil FDA Hydrolase Activity Assay Kit

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

Operation Equipment: Microplate Reader/Spectrophotometer

Cat No: NA0587

Size:100T/48S

Components:

Reagent I: 50 mL×1, storage at 4℃.

Reagent II: Powder×1, storage at -20℃ and protect from light. Add 2 mL of acetone to dissolve before use.

Standard: Powder×1, 10 mg of fluorescein, storage at -20℃ and protect from light. Before use, add 3.03 mL of 50% acetone (acetone(V):distilled water(V)=1:1) to prepare 10 μmol/mL fluorescein standard solution, which can be dissolved in a water bath at 45℃.

Product Description:

Fluorescein diacetate (FDA) hydrolysis reaction is one of the most important biological indicators in the study of soil quality, which can reflect the activity of soil microbial, the change of soil quality and the transformation of organic matter in ecosystem.

FDA is a colorless compound, which can be hydrolyzed by many soil enzymes in the medium. After dehydration reaction, fluorescein is the final product of enzymatic hydrolysis. The fluorescein is stable and not easy to be decomposed, and has a strong absorption peak at 490 nm. The activity of FDA hydrolase can be calculated by detecting the change of absorption value at 490 nm.

Reagents and Equipment Required but Not Provided:

Balance, low temperature centrifuge, spectrophotometer/microplate reader, micro glass cuvette/96 well flat-bottom plate, constant temperature water bath, acetone, 30 mesh sieve (or smaller).

Procedure:

I. Treatment of soil samples:

Natural air drying of fresh soil sample or air drying in 37℃ oven, passing through 30 mesh sieve.

II. Determination steps

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 490 nm, set zero with 50% acetone.

2. Dilute 10 μmol/mL fluorescein standard solution with 50% acetone to 2, 0 μmol/mL standard solution (0 is blank tube). Take 200 μL into the micro glass cuvette/96 well flat-bottom plate to determine the absorbance A_S, A_B at 490 nm separately, calculate $\Delta A_S = A_S - A_B$.

3. Add reagents as the following table.

Reagent name	Control tube (C)	Test tube (T)

Sample (g)	0.03	0.03
Reagent I(μL)	150	150
Acetone (μL)	135	-
Reagent II	15	15
Mix well, shake for 1 hour at 37℃.		
Acetone (μL)	-	135
Centrifuge at 10000 ×g for 5 minutes at 25℃, take 200 μL of the supernatant, measure the absorbance (A) at 490 nm, recorded as A_T, A_C。$\Delta A_T = A_T - A_C$		

Note: The blank tube and standard tube only need to be measured once or twice.

III. The calculation formula of FDA hydrolase activity:

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

FDA (U/g prot) $=(\Delta A \div \Delta A_S \times C_S) \times V_{RT} \div W \div T = 14.4 \times (\Delta A \div \Delta A_S) \div W$

V_{RT}: The total volume of reaction, 0.3 mL;

C_S: Concentration of standard solution, 2 μmol/mL;

T: Catalytic reaction time, 1 hour =1/24 day;

W: Weight of air dried sample, g.

Note:

1. Try to use fresh soil samples or samples preserved under short-term low temperature conditions, otherwise it is difficult to accurately reflect the enzyme activity.

2. Carry out the pre-experiment before the determination. If the absorbance value is greater than 1.2, please carry out the determination to reduce the mass of soil sample, and multiply the dilution multiple in the calculation formula. If the absorption value is too small, it can be determined by increasing the mass of soil sample or reaction time.