Soil FDA Hydrolase Activity Assay Kit

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Operation Equipment: Spectrophotometer

Catalog Number: BC0480

Size:50T/24S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Reagent I	Liquid 30 mL×1	2-8℃
Reagent II	Powder×2	-20℃
Standard	Powder×1	-20℃

Solution Preparation:

1. **Reagent II**: Take a bottle and add 1.5 mL of acetone to dissolve before use. The unused reagent can be stored at - 20℃ for 1 week. Avoid repeated freezing and thawing.
2. **Standard**: 10 mg of fluorescein. 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 45℃ water bath. The unused reagent can be stored at - 20℃ for 2 weeks. Avoid repeated freezing and thawing.

Product Description:

Fluorescein diacetate (FDA) hydrolysis 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.

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment. If the absorption value of the sample is not within the measurement range, it is recommended to dilute or increase the sample size for detection.

Reagents and Equipment Required but Not Provided：

Visible spectrophotometer, 1 mL glass cuvette, balance, desk centrifuge, water bath/constant temperature incubator, acetone (>98%, AR), mortar, 30-50 mesh sieve (or smaller).

Procedure:

I. Sample preparation:

Fresh soil samples are naturally air-dried or oven to dry at 37℃, then sieved by 30 ~ 50 mesh sieve.

Refer to note 1 for soil sample requirements.

II. Determination steps

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

1. Dilution of standard solution: Take 200 μL of 10 μmol/mL fluorescein standard solution, add 800 μL of 50% acetone, mix well, and prepare 2 μmol/mL standard solution for testing (i.e. standard tube); Take 1mL of 50% acetone as the 0 μmol/mL standard solution to be tested (i.e. blank tube), and prepare it on site. (Each tube requires 1 mL in the experiment).
2. Take 1 mL in the 1 mL glass cuvette to determine the absorbance A_S, A_B at 490 nm separately, calculate $\Delta A_S = A_S - A_B$. The blank tube and standard tube only need to be measured once or twice.
3. Sample determination.

Reagent name	Control tube (C)	Test tube (T)
Sample (g)	0.1	0.1
Reagent I (μL)	500	500
Acetone (μL)	450	-
Reagent II (μL)	50	50
Mix thoroughly and place in a 37°C constant temperature water bath or 37°C constant temperature incubator. React accurately for 1 hour, shaking every 10 minutes during this period.		
Acetone (μL)	-	450
Centrifuge at 10000g for 5 minutes at 25°C, take the supernatant and place it in a 1mL glass cuvette, measure the absorbance (A) at 490 nm, recorded as A_T, A_C. $\Delta A = A_T - A_C$.		

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 soil sample) $= (\Delta A \div \Delta A_S \times C_S) \times V_{RT} \div W \div T = 48 \times (\Delta A \div \Delta A_S) \div W$

V_{RT}: The total volume of reaction, 1 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. Conduct a preliminary experiment before measurement. If the absorbance value is greater than 1.2, the soil sample mass can be appropriately reduced before measurement. If the absorbance value is too small, the soil sample quality can be increased or the reaction time can be increased for measurement.

Experimental example:

1. Take two tubes of 0.1g clover soil and place them in 1.5 mL EP tubes, respectively as control tubes

and measurement tubes. Follow the measurement steps and dilute the supernatant 5 times for measurement. Use a 1mL glass cuvette to measure the absorbance value, calculate $\Delta A=A_T-A_C$ =1.148-0.066=1.082, $\Delta A_S=A_S-A_B$=0.499-0=0.499, and calculate the enzyme activity based on soil quality:

FDA activity (U/g soil sample)=48× ($\Delta A \div \Delta A_S$) ÷ W × 5 (dilution ratio)=5204.01 U/g soil sample.

2. Take two tubes of 0.1g forest soil and place them in 1.5mL EP tubes, respectively as control tubes and measurement tubes. Follow the measurement steps and dilute the supernatant 5 times for measurement. Use a 1mL glass cuvette to measure the absorbance value, calculate $\Delta A= A_T-A_C$ =0.973-0.133=0.84,

$\Delta A_S=A_S-A_B$ =0.499-0=0.499, and calculate the enzyme activity based on soil quality:

FDA activity (U/g soil sample)=48 × ($\Delta A \div \Delta A_S$) ÷ W × 5 (dilution ratio)=4040.08 U/g soil sample.

Related publications:

[1] Zhou H, Liu Q, Jiang L, Shen Q, Chen C, Zhang C, Tang J. Enhanced remediation of oil-contaminated intertidal sediment by bacterial consortium of petroleum degraders and biosurfactant producers. Chemosphere. 2023 Jul;330:138763. doi: 10.1016/j.chemosphere.2023.138763. Epub 2023 Apr 22. PMID: 37094722.

[2] Xiang Y, Li S, Rene ER, Xiaoxiu L, Ma W. Enhancing fluoroglucocorticoid defluorination using defluorinated functional strain Acinetobacter. pittii C3 via humic acid-mediated biotransformation. J Hazard Mater. 2022 Aug 15; 436:129284. doi: 10.1016/j.jhazmat.2022.129284. Epub 2022 Jun 6. PMID: 35739793.

[3] Meng X, Wang L, Zhao N, Zhao D, Shen Y, Yao Y, Jing W, Man S, Dai Y, Zhao Y. Stimuli-responsive cancer nanomedicines inhibit glycolysis and impair redox homeostasis. Acta Biomater. 2023 Sep 1; 167:374-386. doi: 10.1016/j.actbio.2023.06.016. Epub 2023 Jun 19. PMID: 37343908.

[4] Wang W, Xie Y, Li H, Dong H, Li B, Guo Y, Wang Y, Guo X, Yin T, Liu X, Zhou W. Responses of lettuce (Lactuca sativa L.) growth and soil properties to conventional

non-biodegradable and new biodegradable microplastics. Environ Pollut. 2024 Jan 15; 341:122897. doi: 10.1016/j.envpol.2023.122897. Epub 2023 Nov 8. PMID: 37949158.

[5] Wang L, Li J, Zhang S. A Comprehensive Network Integrating Signature Microbes and Crucial Soil Properties During Early Biological Soil Crust Formation on Tropical Reef Islands. Front Microbiol. 2022 Mar 17; 13:831710. doi: 10.3389/fmicb.2022.831710. PMID: 35369528; PMCID: PMC8969229.

References：

[1] Sánchez-Monedero M A, Mondini C, Cayuela M L, et al. Fluorescein diacetate hydrolysis, respiration and microbial biomass in freshly amended soils[J]. Biology and Fertility of Soils, 2008, 44(6): 885-890.

[2] Paudel B R, Udawatta R P, Anderson S H. Agroforestry and grass buffer effects on soil quality parameters for grazed pasture and row-crop systems[J]. Applied Soil Ecology, 2011, 48(2): 125-132.

Related Products:

BC0240/BC0245	Soil Saccharase(S-SC) Activity Assay Kit
BC0140/BC0145	Soil Acid Phosphatase(S-ACP) Activity Assay
BC0460/BC0465	Soil Neutral Phosphatase(S-NP) Activity Assay Kit
BC0280/BC0285	Soil Alkaline Phosphatase(S-AKP/ALP) Activity Assay Kit