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CheKine™ Micro Soil Fluorescein Diacetate (S-FDA) Hydrolase Activity Assay Kit

Cat #: KTB4049 Size: 48 T/48 S 96 T/96 S

	Micro Soil Fluorescein Diacetate (S-FDA) Activity Assay Kit		
REF	**Cat #:** KTB4049	LOT	**Lot #:** Refer to product label
	Applicable sample: Soli		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

The hydrolysis reaction of FDA can well reflect the changes of microbial activity and soil quality in soil and the transformation of organic matter in ecosystem, which is one of the important biological indexes in soil quality research. CheKine™ Micro Soil Fluorescein Diacetate (S-FDA) Activity Assay Kit can detect biological samples such as soli. In this kit, FDA is a colorless compound, which can be hydrolyzed by many soil enzymes in the medium, and after dehydration reaction, fluorescein, the final product of enzymatic hydrolysis, is stable and difficult to decompose, and has a strong absorption peak at 490 nm. The activity of S-FDA hydrolase can be calculated by detecting the change of absorption value at 490 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	30 mL	60 mL	4℃
Reagent Ⅱ	Powder×1 vial	Powder×2 vials	4℃, protected from light
Standard	Powder×1 vial	Powder×1 vial	4℃, protected from light

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

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 490 nm
- 96-well microplate or microglass cuvette, precision pipettes, disposable pipette tips,1.5 mL EP tube
- Oscillator, centrifuge, 30-50 mesh sieve
- Deionized water, absolute ethyl alcohol, acetone

Reagent Preparation

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Working Reagent Ⅱ: Prepared before use. Add 2.5 mL acetone to each bottle, dissolve thoroughly. The remaining reagent can

also be stored at -20℃ and protected from light for 1 week after aliquoting to avoid repeated freezing and thawing.

Standard: Prepared before use. Add 3 mL absolute ethyl alcohol to a bottle, dissolve thoroughly, that is 10 μmol/mL luciferin Standard. The remaining reagent can also be stored at -20℃ and protected from light for 2 weeks after aliquoting to avoid repeated freezing and thawing.

0.1 μmol/mL luciferin Standard: Prepare 0.1 μmol/mL luciferin Standard by diluting 10 μL 10 μmol/mL luciferin Standard into 990 μL absolute ethyl alcohol. Using 0.1 μmol/mL luciferin Standard solution for subsequent detection.

Note: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Note: Reagent II or Standard has certain irritation, so personal protection is recommended during use.

Sample Preparation

Note: Note: It is recommended to use fresh soil samples.

Fresh soil samples naturally air dried or air dried in an oven at 37℃ and sieved through 30-50 mesh sieve.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 490 nm, visible spectrophotometer was returned to zero with deionized water.

2. Operation table (The following operations are operated in the 1.5 mL EP tube):

Reagent	Test Tube	Control Tube	Standard Tube	Blank Tube
Sample (g)	0.05	0.05	0	0
Standard (μL)	0	0	40	0
Deionized Water (μL)	0	0	0	40
Reagent I (μL)	200	240	200	200
Reagent II (μL)	40	0	0	0
Mix well, and shake at 30℃ for 1 h.			0	0
Acetone (μL)	160	160	160	160

Mix well, centrifuge at 10,000 g for 5 min at room temperature, take 200 μL into 96-well microplate or microglass cuvette, record the absorbance value at 490 nm. The Blank Well is recorded as A_{Blank}, the Standard Well is marked as $A_{Standard}$, the Control Well is marked as $A_{Control}$, and the Test Well is marked as A_{Test}. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: The Standard Well and Blank Well only need to be done once or twice, Each Test Well needs to be provided with a Control Well. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.01, increase the sample quantity appropriately. If ΔA_{Test} is larger than 1.2, decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

Calculation of the S-FDA hydrolase activity

Active unit definition: The amount of fluorescein produced per gram of soil per day was defined as one unit of enzyme activity.

S-FDA hydrolase (U/g soli)=$C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Reaction}\div W\div T$**=$0.96\times\Delta A_{Test}\div\Delta A_{Standard}\div W$**

$C_{Standard}$: Standard concentration, 0.1 μmol/mL; $V_{Reaction}$: Enzymatic reaction volume 0.4 mL; T: reaction time, 1 h=1/24 d; W: weight of sample, g.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

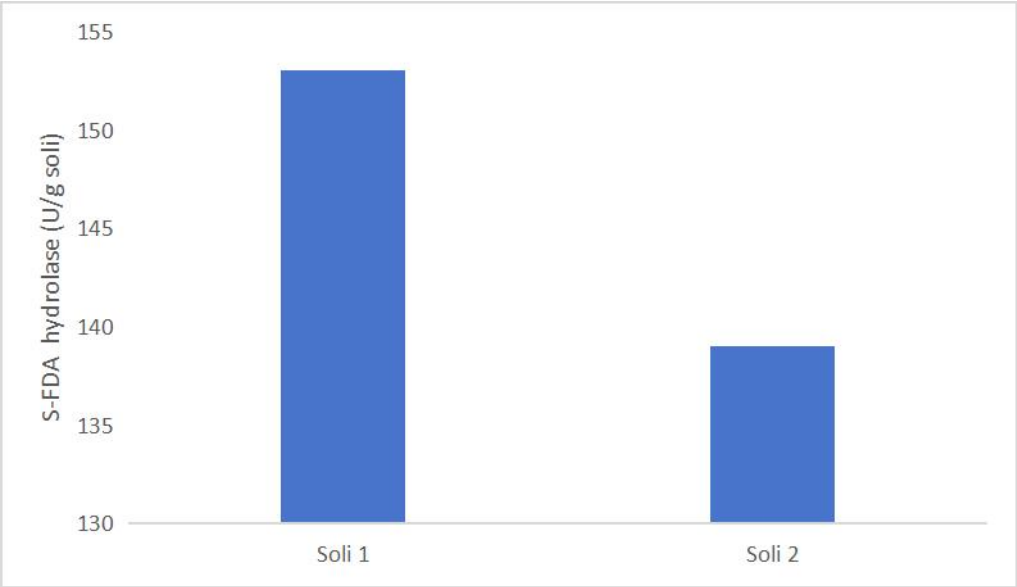

Figure 1. Determination S-FDA hydrolase activity in soli sample by this assay kit.

Recommended Products

Catalog No.	Product Name
KTB4012	CheKine™ Micro Soil Nitrate Nitrogen Assay Kit
KTB4014	CheKine™ Micro Acid Soil Available Phosphorous Assay Kit
KTB4041	CheKine™ Micro Soil Alkaline Phosphatase(S-AKP/ALP) Activity Assay Kit
KTB4050	CheKine™ Micro Soil Catalase (S-CAT) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.