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CheKine™ Micro Soil Laccase (SL) Activity Assay Kit

Cat #: KTB4047

Size: 48 T/48 S 96 T/96 S

[<u>;</u>]	Micro Soil Laccase (SL) Activity Assay Kit		
REF	Cat #: KTB4047	LOT	Lot #: Refer to product label
	Applicable sample: Soli		
X	Storage: Stored at 4°C for 6 months, protected from light		

Assay Principle

Laccase is a kind of copper-containing polyphenol oxidase, belonging to ceruloplasmin oxidase family, which is widely distributed in fungi and higher plants. Laccase has strong redox ability and is widely used in pulp biological bleaching, degradation of environmental pollutants and lignocellulose, and biological detection. CheKine™ Micro Soil Laccase (SL) Activity Assay Kit can detect biological samples such as soli. In this kit, SL decomposes substrate ABTS to produce ABTS free radicals, and the absorption coefficient at 420 nm is much larger than that of substrate ABTS. The SL activity can be calculated by measuring the increase rate of ABTS free radicals.

Materials Supplied and Storage Conditions

Kit componente	Si	Storage conditions		
Kit components	48 T	96 T	Storage conditions	
Reagent	50 mL	100 mL	4°C	
Reagent II	Powder×1 vial	Powder×2 vials	4°C, 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 420 nm
- 96-well microplate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- · Oscillator, ice maker, freezing centrifuge, 30-50 mesh sieve

Reagent Preparation

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

Working Reagent II: Prepared before use. Add 15 mL Reagent | to each bottle, dissolve thoroughly. The remaining reagent can also be stored at 4°C and protected from light for 1 week. The prepared Working Reagent || should be colorless and transparent. If the reagent changes color, it cannot be used.



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°C 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 420 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
Sample (g)	0.02	0.02
Reagent I (µL)	0	250
Working Reagent II (µL)	250	0

Mix well, shake accurately at 37°C for 10 min, and immediately take an ice bath for 5 min. Centrifuge at 10,000 g for 5 min at 4°C, take 200 μ L supernatant into 96-well microplate or microglass cuvette, record the absorbance value at 420 nm. The Control Well is marked as A_{Control}, and the Test Well is marked as A_{Test}. Finally calculate Δ A=A_{Test}-A_{Control}.

Note: 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 is less than 0.01, increase the sample quantity appropriately. If ΔA is larger than 1.5, the supernatant can be appropriately diluted with Reagent I, the calculated result multiplied by the dilution factor, or 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.

A. 96-well plates calculation formula as below

Active unit definition: The amount of enzyme required to produce 1 nmol ABTS free radical per gram of soil per minute was defined as one unit of enzyme activity.

SL (U /g soli)= $\Delta A \div (\epsilon \times d) \times 10^9 \times V_{Total} \div W \div T=1.39 \times \Delta A \div W$

ε: ABTS free radical molar extinction coefficien, 3.6×10⁴ L/mol/cm; d: 96-well plate diameter, 0.5 cm; V_{Total}: Total reaction volume,

 $0.25 \text{ mL}=2.5 \times 10^{-4} \text{ L}$; W: Sample weight, g; T: reaction time, 10 min; 10^9 : Unit conversion coefficient, 1 mol= $1 \times 10^9 \text{ nmol}$.

B. Microglass cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

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 SL 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.

