



CheKine™ Micro Soil Urease (S-UE) Activity Assay Kit

Cat #: KTB4018

Size: 48 T/24 S

96 T/48 S

	Micro Soil Urease (S-UE) Activity Assay Kit		
REF	Cat #: KTB4018	LOT	Lot #: Refer to product label
	Applicable samples: Soil sample		
	Storage: Stored at 4°C for 6 months, protected from light		

Assay Principle

Soil urease (S-UE) is capable of hydrolyzing urea to produce ammonia and carbonic acid. The activity of soil urease correlates positively with the microbial population, organic matter content, total nitrogen, and available nitrogen content in the soil. Soil urease activity reflects the nitrogen status of the soil. CheKine™ Micro Soil Urease (S-UE) Activity Assay Kit provides a simple, convenient, and rapid method for detecting S-UE activity in soil samples. The detection principle is based on the indophenol blue colorimetric method to measure the NH₃-N produced by urease-catalyzed urea hydrolysis.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent I	Powder×1 vial	Powder×1 vial	4°C
Reagent II	14 mL	28 mL	4°C
Reagent III A	0.3 mL	0.6 mL	4°C, protected from light
Reagent III B	1.2 mL	2.4 mL	4°C, protected from light
Reagent IV	1.5 mL	3 mL	4°C, protected from light
NH ₄ Cl Standard (1 mmol/mL)	1 mL	1 mL	4°C

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 578 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Oven, 30-50 mesh sieve, centrifuge, constant temperature water bath, analytical balance
- Deionized water, toluene

Reagent Preparation

Reagent I : Before use, add 4.5 mL deionized water for 48 T; add 9 mL deionized water for 96 T; Fully dissolve it. Unused reagents should be stored at 4°C for up to two weeks.

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

Reagent III: Before use, pour all Reagent III A into Reagent III B and mix it for use, and store the inexhaustible reagents at 4°C for a week.

Reagent IV: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.

Note: Reagent III A and Reagent IV are toxic and has a pungent odor, so it is recommended to experiment in a fume hood.

Standard Curve Setting: 1 mmol/mL NH₄Cl Standard was diluted to 1 µmol/mL with deionized water. Dilute the 1 µmol/mL NH₄Cl Standard with Extraction Solution to 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.01563 µmol/mL as indicated in the table below.

Num.	Volume of Standard	Volume of Deionized water (µL)	The Concentration of Standard (µmol/mL)
Std.1	400 µL of Standard	0	1
Std.2	200 µL of Std.1 (1 µmol/mL)	200	0.5
Std.3	200 µL of Std.2 (0.5 µmol/mL)	200	0.25
Std.4	200 µL of Std.3 (0.25 µmol/mL)	200	0.125
Std.5	200 µL of Std.4 (0.125 µmol/mL)	200	0.0625
Std.6	200 µL of Std.5 (0.0625 µmol/mL)	200	0.03125
Std.7	200 µL of Std.6 (0.03125 µmol/mL)	200	0.01563
Blank	0	200	0 (Blank Well)

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

Sample Preparation

Note: Fresh samples are recommended.

Fresh soil samples should be air-dried naturally or dried in an oven at 37°C, then passed through a 30-50 mesh sieve.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 578 nm, visible spectrophotometer was returned to zero with deionized water.

2. Enzyme incubation (the following operations are performed in a 1.5 mL centrifuge tube):

Reagent	Test Tube (µL)	Control Tube (µL)
Air-dried soil sample (g)	0.05	0.05
Toluene	20	20
Vortex to mix thoroughly, place 20 min at room temperature		
Reagent I	90	0
Deionized water	0	90
Reagent II	190	190

Mix well, put it in a water bath at 37°C for 24 h, centrifuge at 10,000 g at 25°C for 10 min, and take the supernatant.

3. Dilute the supernatant 10-fold (take 0.1 mL supernatant and add 0.9 mL deionized water).

4. Determination of ammonia (the following operations are performed in 96-well plates or microglass cuvette):

Reagent	Test Well (μL)	Control Well (μL)	Standard Well (μL)
Diluted Supernatant	80	80	0
Standard	0	0	80
ReagentIII	15	15	15
ReagentIV	15	15	15
Mix well and place 20 min at room temperature			
Deionized Water	90	90	90

After rapid mixing, the absorbance value was determined by 578 nm, which was recorded as A_{Test} , A_{Control} , A_{Standard} , A_{Blank} , calculated $\Delta A_{\text{Test}} = A_{\text{Test}} - A_{\text{Control}}$, $\Delta A_{\text{Standard}} = A_{\text{Standard}} - A_{\text{Blank}}$.

Note: The standard curve needs to be determined only once, and a control well should be set up for each measurement 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 0.005, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.5, the sample can be appropriately diluted with Deionized Water, 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.

1. Drawing of standard curve

With the concentration of the Standard Solution as the x-axis and the $\Delta A_{\text{Standard}}$ as the y-axis, draw the standard curve. Substitute the ΔA_{Test} into the equation to obtain the x value (μmol/mL).

2. Calculation of UE activity of samples

Definition of unit: 1 μmol of NH₃-N produced per g of soil sample per d is defined as an enzyme activity unit.

UE (U/g fresh weight) = $x \times 10 \times V_{\text{Total reaction}} \div W \div T = \mathbf{3 \times x \div W}$

Where: 10: Dilution factor; T: Reaction time, 1 d; $V_{\text{Total reaction}}$: Total volume of reaction system, 0.3 mL; W: Sample mass, 0.05 g.

Typical Data

Typical standard curve:

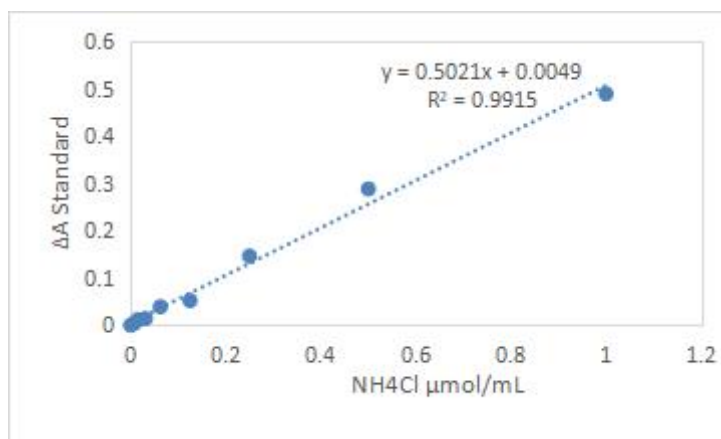


Figure1. Standard Curve for NH₃-N.

Examples:

Take 0.05 g of fresh soil sample that has been dried in a 37°C oven and use 96-well plate to calculate $\Delta A_{\text{Test}} = 0.523 - 0.055 = 0.468$, $x = 0.922$. The content calculated according to the soil sample mass is as follows:

UE (U/g soil sample) = $(0.468 - 0.0049) / 0.5021 = 55.34$ U/g.

Recommended Products

Catalog No.	Product Name
KTB4023	CheKine™ Mirco Soil Peroxidase (S-POD) Activity Assay Kit
KTB4024	CheKine™ Mirco Soil Acid Protease (S-ACPT) Activity Assay Kit
KTB4025	CheKine™ Mirco Soil β -Xylosidase (S- β -XYS) 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.