

Soil Urease (S-UE) Activity Assay Kit

Product Code: 67130

Method: Spectrophotometric Method

Product Introduction

Soil urease (S-UE) hydrolyzes urea to produce ammonia and carbonic acid. Soil urease activity is positively correlated with soil microbial quantity, organic matter content, total nitrogen, and available nitrogen content, and it reflects the nitrogen status of soil.

This kit uses the indophenol blue colorimetric method to determine NH₃-N produced by urease-catalyzed hydrolysis of urea.

Reference example: test sample was 100-fold diluted soil sample supernatant. OD_{630 nm} values were control: 0.049/0.046/0.046; assay: 0.693/0.711/0.704. Actual readings may vary depending on testing instruments and conditions.

Package Contents and Storage

Size: 50T

Code	Item	Amount	Storage
67130.1	Reagent One	Self-prepared	/
67130.2	Reagent Two	2 g	Protected from light, 2-8°C
67130.3	Reagent Three	65 mL	2-8°C
67130.4	Reagent Four	30 mL	Protected from light, 2-8°C
67130.5	Reagent Five	30 mL	Protected from light, 2-8°C
67130.6	Standard	10 mg	2-8°C
67130.m	Instruction Manual	1 copy	/

Quality and Safety Information

Raw Material or Packaging Name	Quality Standard	Main Toxicity
Reagent One	——	——
Reagent II	——	——
Reagent III	——	——
Reagent IV	——	——
Reagent V	——	——
Standard	——	——

Transportation and Storage

Transportation: Transport with ice packs.

Storage: Store according to the instructions. Shelf life: 180 days.

Instructions for Use

1. Sample Processing

Air-dry fresh soil samples naturally or dry them in an oven at 37°C. Pass the dried samples through a 30-50 mesh sieve.

2. Reagent Preparation

- **Reagent I:** Toluene, 10 mL × 1 bottle, stored at 4°C. This reagent is self-prepared.
- **Reagent II:** Before use, add 20 mL distilled water, dissolve completely, and set aside. Store at 4°C. Unused reagent should be stored at 4°C.
- **Standard:** Ammonium chloride, 10 mg. Before use, add 1 mL distilled water to dissolve and prepare a 10 mg/mL standard solution. The solution can be stored at 2-8°C for one month.

3. Operating Steps

1. Preheat the spectrophotometer for 30 min, set the wavelength to 630 nm, and zero with distilled water.
2. Dilute the 10 mg/mL standard solution with distilled water to prepare a 1 mg/mL standard solution: 900 µL distilled water + 100 µL 10 mg/mL standard solution.
3. Dilute the 1 mg/mL standard solution to prepare 50, 25, 12.5, 3.125, 0.78125, and 0.1953125 µg/mL standard solutions for testing.

4. Standard Solution Dilution

No.	Concentration Before Dilution (µg/mL)	Standard Solution Volume (µL)	Distilled Water Volume (µL)	Concentration After Dilution (µg/mL)
1	1000	30	570	50
2	50	300	300	25
3	25	200	200	12.5
4	12.5	100	300	3.125
5	3.125	100	300	0.78125
6	0.78125	100	300	0.1953125

In the following experiment, each standard tube requires 200 µL standard solution. Do not measure absorbance directly in this dilution step.

5. Incubation

Component	Assay Tube	Control Tube
Air-dried soil sample (g)	0.25	0.25
Reagent 1 (µL)	125	125

Shake to mix thoroughly and leave at room temperature for 15 min.

Component	Assay Tube	Control Tube
Reagent 2 (µL)	625	-
Distilled water (µL)	-	625
Reagent 3 (µL)	1250	1250

Mix thoroughly and incubate in a 37°C water bath for 24 h. Then centrifuge at 10000 g and 25°C for 10 min, and collect the supernatant.

6. Supernatant Dilution

Dilute the incubated supernatant 100 times by mixing 0.01 mL supernatant with 0.99 mL distilled water.

7. Ammonia Content Measurement

Component	Assay Tube	Control Tube	Standard Tube	Blank Tube

Diluted supernatant (μL)	200	200	-	-
Standard solution (μL)	-	-	200	-
Distilled water (μL)	-	-	-	200
Reagent 4 (μL)	500	500	500	500
Reagent 5 (μL)	500	500	500	500

Mix thoroughly and let stand at 37°C for 20 min. Transfer 1 mL solution into a 1 mL glass cuvette. Zero with distilled water at 630 nm and measure the absorbance value.

Calculate: $\Delta A = A_{\text{assay tube}} - A_{\text{control tube}}$; $\Delta A_{\text{standard}} = A_{\text{standard tube}} - A_{\text{blank tube}}$.

Set one control tube for each assay tube. The standard curve and blank tube only need to be measured 1-2 times.

Activity Calculation

1. Standard Curve

Use the standard tube concentration (X, μg/mL) and absorbance $\Delta A_{\text{standard}}$ (Y, $\Delta A_{\text{standard}}$) to establish the standard curve. Substitute the sample ΔA value (Y, ΔA) into the standard curve to calculate the sample concentration (X, μg/mL).

2. S-UE Activity

Unit definition: the production of 1 μg NH₃-N per g soil per day is defined as one enzyme activity unit.

S-UE activity (U/g soil sample) = $X \times 100 \times V_{\text{total reaction}} \div W \div T = 800 \times X$

- 100: dilution factor
- T: reaction time, 1 d
- $V_{\text{total reaction}}$: total reaction system volume, 2 mL
- W: sample mass, 0.25 g

Notes

1. Before formal measurement, select 2-3 samples with large expected differences for preliminary testing.
2. Required instruments and supplies: visible spectrophotometer, water bath or metal bath, adjustable pipette, 1 mL glass cuvette, mortar, ice, toluene, and distilled water.
3. This 50T kit can measure 24 samples.
4. The linear range of this kit is 0.1953125-50 μg/mL. If the measured absorbance value is outside the linear absorbance range, increase the sample amount or dilute the sample before measurement. After dilution, multiply by the dilution factor in the calculation formula.

Appendix

For greater accuracy, prepare a standard curve using the operating steps above. A standard curve formula may be used only if the standard curve is established from measured absorbance values and $R^2 \geq 0.99$.

Visual Reference