

# Soil Urease (S-UE) Activity Assay Kit

**Note:** It is necessary to predict 2-3 large difference samples before the formal determination.

**Operation Equipment:** Spectrophotometer/Microplate reader

**Catalog Number:** BC0125

**Size:** 100T/48S

**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	Self-Provided Reagent	-
Reagent II	Powder ×2	2-8°C
Reagent III	Liquid 20 mL×1	2-8°C
Reagent IV A	Liquid 1 mL×1	2-8°C
Reagent IV B	Liquid 4 mL×1	2-8°C
Reagent V	Liquid 0.3 mL×1	2-8°C
Standard	Liquid 1 mL×1	2-8°C

## Solution Preparation:

1. Reagent I: About 2.5mL methylbenzene (Required but not provided), store at RT. A 30mL brown reagent bottle is provided in the kit. Please label the reagent name yourself.
2. Reagent II: Dissolved one Reagent II with 2.7 mL of distilled water before use. The left reagent can be stored at 2-8°C for four weeks.
3. Reagent IV: Mix Reagent IV A: Reagent IV B=1: 4 according to sample number before use.
4. Reagent V: The liquid is placed in an EP tube inside the bottle and needs to be centrifuged before use. Before use, add 5.7 mL of distilled water, mix well and wait for use; The left reagent can be stored at 2-8°C for two weeks.
5. Standard: 1 mg/mL standard solution.

## Product Description

S-UE is an enzyme that catalyzes the hydrolysis of urea into carbon dioxide and ammonia. The microbial quantity of soil, organic matter content, total nitrogen and available nitrogen content have positive correlation with soil urease activity. Soil nitrogen status is determined by soil urease activity. The ammonia is determined by the indophenol blue method, resulting in blue indophenol produced is proportional to the concentration of ammonia.

## Reagents and Equipment Required but Not Provided.

Spectrophotometer/microplate reader, thermostat water bath, transferpette, micro glass cuvette/ 96 well flat-bottom plate, ice, 30-50 mesh sieve (or smaller), methylbenzene (>98%, AR) and distilled water.

## Procedure

### I. Sample processing:

The fresh soil sample shall be dried by naturally or in an oven at 37°C and shall be screened through 30-50 mesh.

### II. Determination procedure:

1. Preheat the spectrophotometer/ microplate reader for more than 30 minutes, adjust the wavelength to 630 nm, set spectrophotometer to zero with distilled water.

#### 2. Sample Preparation

Reagent	Test tube	Contract tube
Air drying soil sample(g)	0.05	0.05
Reagent I (μL)	20	20
Mix thoroughly, wetting all the soil, place at room temperature for 15 minutes.		
Reagent II (μL)	90	-
Distilled water (μL)	-	90
Reagent III (μL)	190	190

Mix thoroughly, culture for 24 hours in 37°C water-bath. Centrifuge at 10000 g for 10 minutes at room temperature. Take the supernatant for test.

3. Dilute the supernatant 10 times. (Add 0.9 mL distilled water to 0.1 mL the supernatant). Dilute until the absorbance less than 1.

4. Prepare standard solution: Diluted the standard to 10, 8, 6, 4, 2, 1, 0.5, 0 μg/mL.

#### 5. Ammonia concentration test

Reagent (μL)	Test tube (T)	Contract tube	Standard
Diluted supernatant solution	120	120	-
Standard solution	-	-	120
Reagent IV	40	40	40
Reagent V	40	40	40
Mixed thoroughly, incubate at room temperature for 20 minutes.			

Mix thoroughly, measure the absorbance at 630 nm which noted as  $A_T$ ,  $A_C$ ,  $A_S$ ,  $A_B$ ,  $\Delta A_T = A_T - A_C$ ,  $\Delta A_S = A_S - A_B$ . A contract tube is required for each test tube. Blank tube and standard curve only need to test once or twice.

### Calculation

1. According to the concentration (x, μg/mL) of the standard tube and the absorbance  $\Delta A_S$  (y,  $\Delta A_S$ ), establish a standard curve. According to the standard curve, bring  $\Delta A_T$  (y,  $\Delta A_T$ ) into the formula to calculate the sample concentration (x, μg/mL)

2. S-UE activity calculation

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1  $\mu\text{g}$  of  $\text{NH}_3\text{-N}$  in the reaction system per day every gram of dry soil sample.

$$\text{S-UE activity (U/g soil sample)} = x \times 10 \times \text{Vrv} \div \text{W} \div \text{T} = 60 \times x$$

10: Dilution factor;

T: Reaction time, 1 day;

Vrv: Total reaction volume, 0.3 mL;

W: Sample weight, 0.05 g.

### Recent Product Citations:

[1] Fazal A, Yang M, Wang X, Lu Y, Yao W, Luo F, Han M, Song Y, Cai J, Yin T, Niu K, Sun S, Qi J, Lu G, Wen Z, Yang Y. Discrepancies in rhizobacterial assembly caused by glyphosate application and herbicide-tolerant soybean Co-expressing GAT and EPSPS. *J Hazard Mater.* 2023 May 15; 450:131053. doi: 10.1016/j.jhazmat.2023.131053. Epub 2023 Feb 22. PMID: 36842198.

[2] Fazal A, Wen Z, Yang M, Wang C, Hao C, Lai X, Jie W, Yang L, He Z, Yang H, Cai J, Qi J, Lu G, Niu K, Sun S, Yang Y. Triple-transgenic soybean in conjunction with glyphosate drive patterns in the rhizosphere microbial community assembly. *Environ Pollut.* 2023 Oct 15; 335:122337. doi: 10.1016/j.envpol.2023.122337. PMID: 37562532.

[3] Yang M, Luo F, Song Y, Ma S, Ma Y, Fazal A, Yin T, Lu G, Sun S, Qi J, Wen Z, Li Y, Yang Y. The host niches of soybean rather than genetic modification or glyphosate application drive the assembly of root-associated microbial communities. *Microb Biotechnol.* 2022 Dec;15(12):2942-2957. doi: 10.1111/1751-7915.14164. Epub 2022 Nov 6. PMID: 36336802; PMCID: PMC9733649.

[4] Liu S, Sui Y, Dong B. Reinforcement of reclaimed sand by stimulating native microorganisms for biomineralization. *Front Bioeng Biotechnol.* 2022 Dec 22; 10:1050694. doi: 10.3389/fbioe.2022.1050694. PMID: 36619391; PMCID: PMC9813752.

### References:

[1] Kandeler E, Gerber H. Short-term assay of soil urease activity using colorimetric determination of ammonium[J]. *Biology and fertility of Soils*, 1988, 6(1): 68-72.

[2] Witte C P, Medina-Escobar N. In-gel detection of urease with nitroblue tetrazolium and quantification of the enzyme from different crop plants using the indophenol reaction[J]. *Analytical biochemistry*, 2001, 290(1): 102-107.

[3] Guo H, Yao J, Cai M, et al. Effects of petroleum contamination on soil microbial numbers, metabolic activity and urease activity[J]. *Chemosphere*, 2012, 87(11): 1273-1280.

Related Products:

### Related Products:

BC0110/BC0115 Soil Polyphenoloxidase (S-PPO) Activity Assay Kit

BC0280/BC0285 Soil Alkaline Phosphatase(S-AKP/ALP) Activity Assay Kit

BC4040/BC4045 Soil Neutral Invertase(S-NI) Activity Assay Kit

