

Soil Urease(UE) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: AK0592

Size: 50T/24S

Components:

Reagent I: Liquid 10 mL×1. Methylbenzene. Storage at 4°C . Required but not provided.

Reagent II : Powder ×1. Dissolved with 20 mL of distilled water before use. Storage at 4°C .

Reagent III: Liquid 65 mL×1. Storage at 4°C .

Reagent IV Solution A: Liquid 2 mL×1. Storage at 4°C .

Reagent IV Solution B: Liquid 8 mL×1. Storage at 4°C . Before using, mix Solution A and Solution B according to the volume ratio of 1:4 for use; mix the amount with how much.

Reagent V : Liquid 0.5 mL×1. Storage at 4°C . The liquid is placed in the EP tube in the reagent bottle. Before use, add 9.5 mL of distilled water, mix well and wait for use; store the reagent that cannot be used up at 4°C;

Standard: Liquid 1 mL×1, 1 mg/mL standard solution.

Product Description

Urease 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, thermostat water bath, transferpettor, 1 mL glass cuvette, ice, 30-50 mesh sieve (or smaller), distilled water, Methylbenzene (Express delivery not allowed), 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 for more than 30 minutes, adjust the wavelength to 630 nm, set zero with distilled water.
2. Sample Preparation

Reagent	Test tube	Contract tube
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Air drying soil sample (g)	0.25	0.25
Reagent I (μL)	125	125
Mix thoroughly, wetting all the soil, place at room temperature for 15 minutes.		
Reagent II (μL)	625	-
Distilled water (μL)	-	625
Reagent III (μL)	1250	1250
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.		

- Dilute the supernatant 10 times. (Add 0.9 mL of distilled water to 0.1 mL of the supernatant). Dilute until the absorbance less than 1.5.
- Prepare standard solution: Diluted the standard to 8, 6, 4, 2, 1, 0.5, 0.25, 0 μg/mL.
- Ammonia concentration test

Reagent	Test tube(T)	Contract tube(C)	Standard tube
Diluted supernatant solution/ Standard solution	360	360	360
Reagent IV (μL)	120	120	120
Reagent V (μL)	120	120	120
Mix thoroughly, incubate at room temperature for 20 minutes.			
Distilled water (μL)	400	400	400

Mix thoroughly, set the counter to zero with distilled water at 630 nm and measure the absorbance which noted as A, $\Delta A = A(T) - A(C)$. Set a contract cube for each test tube.

Make standard curve: Get the standard curve according to standard concentration (y) and absorbance (x, subtract the blank).

Calculation

According to the standard curve, take $\Delta A(x)$ into the formula to get the concentration (μg/mL) of sample(y).

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 μg of NH₃-N in the reaction system per day every gram of dry soil sample.

$$\text{Urease activity (U/g soil sample)} = y \times 10 \times V_{rv} \div W \div T = 80 \times y$$

10: Dilution factor;

T: Reaction time, 1 day;

V_{rv}: Total reaction volume, 2 mL;

W: Sample weight ,0.25 g.

Recent Product Citations:

[1] Hou Q, Wang W, Yang Y, et al. Rhizosphere microbial diversity and community dynamics during potato cultivation[J]. European Journal of Soil Biology, 2020, 98: 103176.

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:

AK0594/AK0593 Soil Polyphenoloxidase (S-PPO) Activity Assay Kit

AK0566/AK0565 Soil Alkaline Phosphatase(S-AKP/ALP) Activity Assay Kit

AK0116/AK0115 Soil Neutral Invertase(S-NI) Activity Assay Kit