

Soil Uricase Activity Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Cat No: BC4415

Size: 100T/48S

Components:

Reagent I: Toluene 2 mL×1, required but not provided, storage at 4°C.

Reagent II A: Liquid 0.5 mL×1, storage at 4°C and protect from light.

Reagent II B: Liquid 17.5 mL×1, storage at 4°C.

Reagent III: Liquid 40 mL×1, storage at 4°C.

Reagent IV: Liquid 50 mL×1, storage at 4°C.

Standard: Liquid 1 mL×1, 5 μmol/mL of uric acid standard solution, storage at 4°C and protect from light.

Preparation of Reagent II: Before use, Reagent II A and Reagent II B are mixed in a 1: 35 ratio, prepared according to sample size.

Product Description:

Soil urase is a kind of oxidoreductase related to nucleic acid metabolism. It mainly converts nucleic acid adenine and uric acid in the soil into allantoin and allantoic acid, and then generates urea for use by plants.

Soil urase can catalyze the production of allantoin, CO₂ and H₂O₂ by uric acid. Uric acid has a characteristic absorption peak at 284 nm. The soil uric acid activity is measured by measuring the amount of uric acid decrease before and after the reaction.

Reagents and Equipment Required but Not Provided:

Ultraviolet spectrophotometer, low temperature centrifuge, constant temperature water bath, mortar, transferpettor, micro quartz cuvette/96 UV well plate, 30-50 mesh sieve, toluene, ice and distilled water.

Sample preparation:

The fresh soil sample is air-dried naturally or in an oven at 37°C, and passed through a 30-50 mesh sieve.

Determination steps:

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 284 nm, set spectrophotometer counter to zero with distilled water.
2. Dilute 5 μmol/mL of uric acid standard solution with distilled water to 1, 0.5, 0.25, 0.125, 0.0625, 0.03125 and 0.015625 μmol/mL standard solution
3. Add reagents as the following table.

Reagent	Test tube (T)	Contrast tube (C)	Soilless tube (So)	Standard tube (St)	Blank tube (B)
Air-dried soil sample (g)	0.05	0.05	-	-	-
Reagent I (μL)	12.5	12.5	12.5	-	-
Shake for make the soil sample completely wet, incubate in room temperature for 30 minutes.					
Reagent II (μL)	250	-	250	-	-
Distilled water (μL)	250	250	250	-	-
Reagent III (μL)	250	500	250	-	-
Shake for mix well, incubate at 30°C for 24 hours. Centrifuge at 10000 rpm for 10 minutes at 25°C, take the supernatant for test.					
Supernatant (μL)	60	60	60	-	-
Standard solution (μL)	-	-	-	60	-
Distilled water (μL)	-	-	-	-	60
Reagent IV (μL)	340	340	340	340	340

Mix thoroughly, take 200 μL of the reaction solution in micro quartz cuvette/96 UV well plate. Detect the absorbance at 284 nm, record as A_T , A_C , A_{So} , A_{St} and A_B respectively. $\Delta A = (A_{So} - A_B) - (A_T - A_C)$, $\Delta A_{St} = A_{St} - A_B$.

Note: A control tube is required for each test tube. Testing of the same batch of samples, the soilless tube and blank tube only need to be measured once or twice. Standard curve only need to be measured once or twice.

III. The calculation formula of soil uricase activity:

1. Create standard curve

Using the concentration of standard solution as x axis and ΔA_{St} as y axis create standard curve, obtain equation $y=kx+b$. Put ΔA into the equation and obtain the x (μmol/mL).

2. Calculation of soil uricase activity.

Unit definition: One unit of enzyme activity is defined as that per gram of soil sample hydrolyze 1 μmol of uric acid per day.

$$\text{Soil uricase activity (U/g soil sample)} = x \times V_{RT} \div W \div T = 0.7625x \div W$$

V_{RT} : The total volume of reaction, 0.7625 mL;

T: Catalytic reaction time, 1 day = 24 hour;

W: Weight of air dried sample, 0.05 g.

Experimental Examples:

1. Take 0.05g of two tubes of No. 2-1-20 soil sample, and carry out the determination according to the operation steps. The calculation is: $\Delta A = (A_{So} - A_B) - (A_T - A_C) = (1.0928 - 0.0023) - (0.6852 - 0.0509) = 0.4562$, Bring into the standard curve $y = 1.6894x + 0.0016$, $x = 0.2691$, calculate the enzyme activity:

$$\text{Soil Uricase Activity (U/g soil sample)} = 0.7625x \div W = 0.7625 \times 0.2691 \div 0.05 = 4.1 \text{ U/g soil sample}$$