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Soil Uricase Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/ microplate reader

Cat No: AK0066

Size:100T/48S

Components:

Reagent I: Toluene 2 mL×1, storage at 4℃ . Toluene need provide for yourself.

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

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

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

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

Standard: Liquid 1 mL×1, 5 μmol/mL of uric acid standard solution, storage at 4℃ 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, CO_2 and H_2O_2 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：

Balance, low temperature centrifuge, spectrophotometer/microplate reader, micro quartz cuvette/96 UV well plate, constant temperature water bath, toluene, 30 mesh sieve(or smaller).

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 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 25C for 24 hours. Centrifuge at 10000 rpm for 10 minutes at 25C, 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 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.

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

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

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

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: ΔA= (Aso-Ab)- (At-Ac)= (1.0928-0.0023)- (0.6852-0.0509) =0.4562, Bring into the standard curve y=1.6894x + 0.0016，计算 x=0.2691, calculate the enzyme activity:

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

Related Products:

AK0592/AK0591	Soil Urease(UE) Activity Assay Kit
AK0596/AK0595	Soil Catalase(S-CAT) Activity Assay Kit
AK0508/AK0507	Soil Peroxidase Activity Assay Kit

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

AK0370/AK0369 Soil Nitrate Reductase(NR) Activity Assay Kit