Soil Chitinase 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: NA0489

Size: 100T/48S

Components:

Reagent I: Liquid 3 mL×1，Toluene needs to be prepared by yourself. Storage at room tempareture.

Reagent II: Liquid 20 mL×1. Storage at 4℃.

Reagent III: Liquid 25 mL×1. Storage at 4℃. It is suspension, shake well before use.

Reagent IV: Liquid 3 mL×1. Storage at 4℃.

Reagent VA: Powder×3. Storage at 4℃.

Reagent VB: Liquid 40 mL×1. Storage at 4℃.

Before use, add 12 mL of Reagent VB into a bottle of Reagent VA, dissolve it completely, and use it now.

Standard solution: 5 mg of N-acetylglucosamine. Before use, add 1 mL of Reagent II to prepare 5 mg/mL standard solution, that is 5000 μg/mL standard solution.

Product Description:

Chitin is the second largest class of biopolymers in nature, which is second only to cellulose. Due to its slow decomposition and large accumulation, it is easy to cause serious environmental pollution. Chitinase is an important enzyme affecting nitrogen mineralization in soil, and its decomposition of chitin controls the key step of nitrogen cycle.

Chitinase hydrolyzes chitin to produce N-acetylglucosamine. The intermediate compound produced by the reaction of N-acetylglucosamine and alkali can further react with p-Dimethylaminobenzaldehyde to produce a chromogenic substance. The chromogenic substance has a characteristic absorption peak at 585 nm. The increasing rate of absorbance reflects the activity of chitinase.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, Low tempareture centrifuge, transferpettor, oscillator, micro glass cuvette/96 well flat-bottom plate, mortar, 30-50 mesh sieve, distilled water and toluene.

Procedure:

I. Sample preparation (the sample size can be adjusted appropriately, and the specific proportion can be referred to the literature):

Air dry the fresh soil sample and sift through 30-50 mesh. Weigh about 0.1g soil sample, add 0.05 mL Reagent I, mix well, and stand at room temperature for 15 min. After standing, add 0.2 mL of Reagent II and 0.4 mL of Reagent III, vibrate at 37℃ for 24 h, rotate at 8000 rpm, and centrifuge at 4℃ for 10 min (if there are still impurities in the supernatant after centrifugation, it is recommended to centrifuge the

supernatant again until it is clear). Take the supernatant and place it on ice for testing.

II. Determination procedure:

1. Preheat the Spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 585 nm, set zero with distilled water.
2. Add reagents with the following list:

Reagent (μL)	Control (C)	Test tube (T)	Blank tube (B)	Standard (S)
Supernatant	100	100	-	-
Reagent II	-	-	100	-
Standard solution	-	-	-	100
Reagent IV	20	20	20	20
	Mix well and stand at room temperature for 5 min	Mix well, bath in boiling water for 5 min, cool with running water to room temperature		
Reagent V	300	300	300	300
Mix well, take water bath at 37℃ for 20 min, absorb 200 μL reaction solution and measure the absorbance value at 585 nm in micro glass cuvette/96 well plate, and record as A_C, A_T, A_B and A_S respectively. Calculate $\Delta_A = A_D - A_C$, $\Delta A_S = A_S - A_B$.				

III. Calculation:

Definition of enzyme activity: at 37℃, the amount of enzyme that decomposes chitin to produce 1 μg N-acetylglucosamine per gram of soil per day is one enzyme activity unit.

Soil chitinase activity (U/g soil sample) = $(\Delta A \div \Delta A_S \times C_S) \times V \div W \div T = 40.625 \times \Delta A \div \Delta A_S \div W$

C_S: concentration of standard tube, 62.5 μg/mL; V: total volume of sample treatment, 0.65 mL; W: sample mass, g; T: reaction time, 1d.

Note:

1. Colorimetry is carried out immediately after the reaction.
2. As soon as the Reagent IVis taken out from the low temperature (4℃), there will be crystal precipitation, which can be dissolved by heating.
3. Reagent V has certain toxicity, so personal protection should be done during the test operation.
4. When A is greater than 1, it is recommended to dilute the sample supernatant with Reagent II.

Experimental examples:

1. Take 0.1 g of soil sample 1 and operate according to the determination steps. After measuring the data with 96 well plate, calculate $\Delta A = A_T - A_C = 0.098-0.051 = 0.047$, $\Delta A_S = A_S - A_B = 0.612-0.049 = 0.563$. The enzyme activity was calculated according to the formula.

Soil chitinase activity (U/g soil sample) = $40.625 \times \Delta A \div \Delta A_S \div W = 40.625 \times 0.047 \div 0.563 \div 0.1 = 33.9$ U/g soil sample.

Related products:

NA0859/NA0403 Soil cellulase (S-CL) activity assay kit

NA0846/NA0604 Soil alkaline phosphatase (S-AKP/ALP) activity assay kit

NA0644/NA0402 Soil nitrate reductase (S-MR) activity assay kit