

Soil Chitinase Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: BC1930

Size: 50T/24S

Components:

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

Reagent II: Liquid 15 mL×1. Storage at 4°C.

Reagent III: Liquid 18 mL×1. Storage at 4°C. It is suspension, shake well before use.

Reagent IV: Liquid 4 mL×1. Storage at 4°C. As soon as the Reagent IV is taken out from the low temperature (4°C), there will be crystal precipitation, which can be dissolved by heating.

Reagent VA: Powder×2. Storage at 4°C.

Reagent VB: Liquid 55 mL×1. Storage at 4°C. Before use, add 25 mL of Reagent VB into a bottle of Reagent VA, dissolve it completely, and use it now. Unused reagents can be stored at 4°C for 4 weeks.

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. Then double dilute 125 times with reagent II to obtain 40µg/mL standard solution to be used. Unused reagents can be stored at 4°C for 4 weeks.

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, low temperature centrifuge, transferpettor, oscillator, 1 mL glass cuvette, mortar, 30-50 mesh sieve (or smaller), distilled water and toluene, water bath or constant temperature incubator.

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 0.15g soil sample, add 0.075 mL Reagent I, mix well, and stand at room temperature for 15 min. After standing, add 0.3

mL of Reagent

II and 0.6 mL of Reagent III, vibrate at 37°C for 24 h, centrifuge at 8000 rpm, 4°C 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 for testing.

II. Determination procedure:

1. Preheat the Spectrophotometer 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	250	250	-	-
Reagent II	-	-	250	-
Standard solution	-	-	-	250
Reagent IV	50	50	50	50
	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	750	750	750	750

Mix well, take water bath or constant temperature incubator at 37°C for 20 min, absorb 1 mL reaction solution and measure the absorbance value at 585 nm in 1 mL glass cuvette, and record as A_C , A_T , A_B and A_S respectively. Calculate $\Delta A = A_T - A_C$, $\Delta A_S = A_S - A_B$. The standard tube and blank tube only need to be measured 1-2 times.

III. Calculation:

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

$$\text{Soil chitinase activity (U/g soil sample)} = (\Delta A \div \Delta A_S \times C_S) \times V \div W \div T = 39 \times \Delta A \div \Delta A_S \div W$$

C_S : concentration of standard tube, 40 μg/mL; V : total volume of sample treatment, 0.975 mL; W : sample mass, g; T : reaction time, 1d.

Note:

1. Colorimetry is carried out immediately after the reaction.
2. Reagent V has certain toxicity, so personal protection should be done during the test operation.
3. When A is greater than 1, it is recommended to dilute the sample supernatant with Reagent II.

Experimental examples:

1. Take 0.15 g of soil sample 1 and operate according to the determination steps. After measuring the data with 1 mL glass cuvette, calculate $\Delta A = A_T - A_C = 0.099 - 0.010 = 0.089$, $\Delta A_S = A_S - A_B = 0.705 - 0.009 = 0.696$. The enzyme activity was calculated according to the formula.

Soil chitinase activity (U/g soil sample) = $39 \times \Delta A \div \Delta A_S \div W = 39 \times 0.089 \div 0.696 \div 0.15 = 33.2$ U/g soil sample.

Related products:

BC0150/BC0155 Soil cellulase (S-CL) activity assay kit

BC0280/BC0285 Soil alkaline phosphatase (S-AKP/ALP) activity assay kit

BC3100/BC3105 Soil nitrate reductase (S-MR) activity assay kit