

Soil N-Acetyl- β -D-Glucosidase (S-NAG) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/ Microplate Reader

Catalog Number: NA0365

Size:100T/48S

Components:

Reagent I: Liquid 30 mL \times 1. Storage at 4°C.

Reagent II: Powder \times 1. Storage at -20°C. Dissolve with 5 mL of distilled water before use. The left reagent store at -20°C.

Reagent III: Liquid 30 mL \times 1. Storage at 4°C.

Standard: Liquid 1 mL \times 1. Storage at 4°C. 5 mmol/L Phenol standard solution. Dilute the standard solution for 50 times to 100 μ mol/L with the Reagent I before use.

Product Description:

Soil N-acetyl- β -D-glucosidase(S-NAG) is an acid hydrolase in lysosomes secreted by soil microorganisms. The activity of S-NAG is closely related to some pathological condition of the body.

S-NAG can catalyze the 4-Nitrophenyl-N-acetyl- β -D-glucopyranoside to p-nitrophenol. The product has characteristic of absorption at 400 nm. In this kit, the S-NAG activity is quantified by measuring the increase in the color development at 400 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/ Microplate Reader, water-bath, desk centrifuge, transferpettor, micro glass cuvette/96 well flat bottom plate, analytical balance, mortar, 30 mesh screen, ice and distilled water.

Procedure:

I. Preparation of samples

Fresh soil samples are naturally air-dried or oven-dried at 37°C, pass through a 30-50 mesh sieve.

II. Determination procedure:

1. Preheat spectrophotometer/microplate reader or spectrophotometer for 30 minutes, adjust the wavelength to 400 nm, set zero with distilled water.
2. Add reagents with the following list:

Reagent	Test tube (T)	Contrast Tube (C)	Standard tube (S)	Blank tube (B)
Air-dried soil (g)	0.03	0.03	-	-
Reagent I (μ L)	142	142	-	-
Reagent II (μ L)	38	-	-	-

Mix thoroughly and incubate the reaction for 60 minutes at 37°C water bath, then take the reaction solution in a boiling water bath for 5 minutes immediately (tightly close to prevent moisture loss), flowing water to cool.				
Reagent II (μL)	-	38	-	-
Mix thoroughly, centrifuge at 10000 ×g for 10 minutes 25°C and take the supernatant.				
Supernatant (μL)	100	100	-	-
Standard solution (μL)	-	-	100	-
Distilled water (μL)	-	-	-	100
Reagent III (μL)	200	200	200	200

Mix thoroughly and stand at room temperature for 2 minutes. **Take 200 μL supernatant and put it in a micro glass cuvette or directly in a 96 well plate.** Detect the absorbance of each tube at 400nm and noted as A_T , A_C , A_S and A_B . Calculate $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$. Each test tube should be provided with one contrast tube.

III. S-NAG activity calculation:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the generation 1μmol of p-nitrophenol every gram of soil sample in the reaction system per day.

$$\text{S-NAG (U/g soil sample)} = \Delta A_T \div (\Delta A_S \div C) \times V_{rv} \div W \div T = 0.432 \times \Delta A_T \div \Delta A_S \div W$$

C: **Concentration of standard solution**, 100 μmol/L;

V_{rv} : Total volume in catalyze system, 1.8×10^{-4} L;

W: Soil sample weight, g;

T: Reaction time, 1 hour = 1/24 day;

Note:

1. If the $\Delta A_T > 1$, the supernatant can be determined after being appropriately diluted. If the $\Delta A_T < 0.02$, the supernatant can be determined after extending the response time. When calculation, multiply the calculation formula by the corresponding dilution factor or change the response time.

Experimental Examples:

1. Take two tubes of 0.03 g soil, which are the measuring tube and the control tube. Follow the measuring steps and mark them as A_t and A_c . Calculate $\Delta A_t = A_t - A_c = 0.305 - 0.271 = 0.034$, $\Delta A_s = A_s - A_b = 0.412 - 0.046 = 0.366$, calculate the enzyme activity:

$$\text{S-NAG activity (U/g soil)} = 0.432 \times \Delta A_t \div \Delta A_s \div W = 0.432 \times 0.034 \div 0.366 \div 0.03 = 1.3377 \text{ U/g soil.}$$

2. Take two tubes of 0.03g forest soil samples, which are the measuring tube and the control tube. Follow the measuring steps and mark them as A_t and A_c . Calculate $\Delta A_t = A_t - A_c = 0.325 - 0.278 = 0.047$, $\Delta A_s = A_s - A_b = 0.412 - 0.046 = 0.366$, calculate enzyme activity:

$$\text{S-NAG activity (U/g soil)} = 0.432 \times \Delta A_t \div \Delta A_s \div W = 0.432 \times 0.047 \div 0.366 \div 0.03 = 1.8492 \text{ U/g soil}$$

Related Products:

NA0364/NA0363 Soil β-Xylosidase(S-β-XYS) Activity Assay Kit

NA0645/NA0404 Soil α-glucosidase(S-α-GC) Activity Assay Kit

NA0850/NA0608 Soil Saccharase(S-SC) Activity Assay Kit

