

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

Catalog Number: AK0124

Size:50T/24S

Components:

Reagent I: Liquid 40 mL \times 1. Storage at 4C.

Reagent II : Powder \times 1. Storage at -20C .Dissolve with 7.5 mL of distilled water before use. The left reagent store at -20C.

Reagent III: Liquid 60 mL \times 1. Storage at 4C.

Standard: Liquid 1 mL \times 1. Storage at 4C. 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, water-bath, desk centrifuge, transferpettor, 1 mL glass cuvette, analytical balance, mortar, 30 mesh sieve (or samller), ice and distilled water.

Procedure:

I. Preparation of samples

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

II. Determination procedure:

1. Preheat Spectrophotometer for 30 minutes, adjust the wavelength to 400 nm, set zero with distilled water.

2. Add reagents in 1 mL glass cuvette as the following:

| Reagent | Test tube (T) | Contrast Tube (C) | Standard tube (S) | Blank tube (B) |
|-----------------------|---------------|-------------------|-------------------|----------------|
| Air-dried soil (g) | 0.1 | 0.1 | - | - |
| Reagent I (μ L) | 475 | 475 | - | - |
| Reagent II (μ L) | 125 | - | - | - |

Mix thoroughly and incubate the reaction for 60 minutes at 37C 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.

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|--|------|------|------|------|
| Reagent II (μL) | - | 125 | - | - |
| Mix thoroughly, centrifuge at 10000×g for 10 minutes 25C and take the supernatant. | | | | |
| Supernatant (μL) | 500 | 500 | - | - |
| Standard solution (μL) | - | - | 500 | - |
| Distilled water (μL) | - | - | - | 500 |
| Reagent III (μL) | 1000 | 1000 | 1000 | 1000 |

Mix thoroughly and stand at room temperature for 2 minutes. 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 ofp-nitrophenol every gram of soil sample in the reaction system per day.

$$\text{S-NAG (U/g soil sample)} = \Delta A_T \div (\Delta A_S \div C) \times V_{rv} \div W \div T = 1.44 \times \Delta A_T \div \Delta A_S \div W$$

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

V_{rv} : Total volume in catalyze system, 6×10^{-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 0.1g clover soil, which are the measuring tube and the control tube. Follow the measuring steps and mark them as A_t and A_c . Calculate $\Delta A_t = A_t - A_c = 0.509 - 0.434 = 0.075$, $\Delta A_s = A_s - A_b = 0.604 - 0.002 = 0.602$, calculate the enzyme activity:

$$\text{S-NAG activity (U/g soil)} = 1.44 \times \Delta A \div \Delta A_s \div W = 1.44 \times 0.075 \div 0.602 \div 0.1 = 1.79402 \text{ U/g soil.}$$

2. Take two tubes of 0.1g 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 $\Delta A_t = A_t - A_c = 0.574 - 0.497 = 0.077$, $\Delta A_s = A_s - A_b = 0.604 - 0.002 = 0.602$, calculate enzyme activity:

$$\text{S-NAG activity (U/g soil)} = 1.44 \times \Delta A \div \Delta A_s \div W = 1.44 \times 0.077 \div 0.602 \div 0.1 = 1.84186 \text{ U/g soil}$$

Related Products:

| | |
|---------------|---|
| AK0122/AK0121 | Soil β-Xylosidase(S-β-XYS) Activity Assay Kit |
| AK0155/AK0154 | Soil α-glucosidase(S-α-GC) Activity Assay Kit |
| AK0574/AK0573 | Soil Saccharase(S-SC) Activity Assay Kit |