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: BC4005

Size:100T/48S

Components:

Reagent I: Liquid 30 mL×1. Storage at 4℃.

Reagent II: Powder×2. Storage at -20℃. Before use, take 1 bottle and add 2.5 mL of distilled water to dissolve it, and the unused reagent can be aliquoted and stored at -20℃ for 4 weeks

Reagent III: Liquid 30 mL×1. Storage at 4℃.

Standard: Liquid 1 mL×1. Storage at 4℃. 5 mmol/L Phenol standard solution.

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 Constant temperature incubator, 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℃, 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, spectrophotometer set zero with distilled water.
2. Dilution of standard solution: Take 20μL of 5 mmol/L p-nitrophenol solution before use, add 980μL of reagent I, mix well, and make a 100 μmol/L standard solution for use, ready to use. (In the experiment, each tube needs 100μL, in order to reduce the experiment error, so prepare a large volume.)
3. 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 (μL)	142	142	-	-

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Reagent II (μL)	38	-	-	-
Mix thoroughly and incubate the reaction for 60 minutes at 37℃ 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 12000 ×g for 10 minutes 25℃ and take the supernatant.				
Supernatant (μL)	100	100	-	-
Standard solution (μL)	-	-	100	-
Distilled water (μL)	-	-	-	100
Reagent III (μL)	200	200	200	200

After standing for 2 minutes at room temperature, centrifuge at 10000g room temperature for 5 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.

S-NAG (U/g soil sample) $=\Delta A_T \div (\Delta A_S \div C) \times Vrv \div W \div T = 0.432 \times \Delta A_T \div \Delta A_S \div W$

C: Concentration of standard solution, 100 μmol/L;
Vrv: 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 At and Ac. Measure and calculate with 96-well plate ΔAt=At-Ac=0.305-0.271=0.034, ΔAs=As-Ab=0.412-0.046=0.366, calculate the enzyme activity:
   S-NAG activity (U/g soil)= =0.432×ΔA÷ΔAs÷W=0.432×0.034÷0.366÷0.03=1.3377U/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 At and Ac. Measure and calculate with 96-well plate ΔAt=At-Ac=0.325-0.278=0.047, ΔAs=As-Ab=0.412-0.046=0.366, calculate enzyme activity:
   S-NAG activity (U/g soil) =0.432×ΔA÷ΔAs÷W=0.432×0.047÷0.366÷0.03=1.8492U/g soil

Related Products:

BC4010/BC4015 Soil β-Xylosidase(S-β-XYS) Activity Assay Kit

BC3080/BC3085 Soil α-glucosidase(S-α-GC) Activity Assay Kit

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