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: BC4000

Size:50T/24S

Components:

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

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

Reagent III: Liquid 60 mL×1. Storage at 4℃.

Standard: Liquid 1 mL×1. Storage at 4℃. 5 mmol/L p-nitrophenol 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, water-bath/ Constant temperature incubator, desk centrifuge, transferpettor, 1 mL glass cuvette, 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 37℃, 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. 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 500μL, in order to reduce the experiment error, so prepare a large volume.)
3. 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 (μL)	475	475	-	-
Reagent II (μL)	125	-	-	-

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)	-	125	-	-
Mix thoroughly, centrifuge at 12000×g for 10 minutes 25℃ and take the supernatant.				
Supernatant (μL)	500	500	-	-
Standard solution (μL)	-	-	500	-
Distilled water (μL)	-	-	-	500
Reagent III (μL)	1000	1000	1000	1000

After standing for 2 minutes at room temperature, centrifuge at 10000g room temperature for 5 minutes, take the supernatant and measure the absorbance A 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) $= \Delta A_T \div (\Delta A_S \div C) \times Vrv \div W \div T = 1.44 \times \Delta A_T \div \Delta A_S \div W$

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

Vrv: 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 At and Ac. 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:
   S-NAG activity (U/g soil sample)$= 1.44 \times \Delta A_T \div \Delta As \div W = 1.44 \times 0.075 \div 0.602 \div 0.1 = 1.79402$ U/g.
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 At and Ac. 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:
   S-NAG activity (U/g soil sample)$= 1.44 \times \Delta A_T \div \Delta A\ s \div W = 1.44 \times 0.077 \div 0.602 \div 0.1 = 1.84186$ U/g.

Related Products:

BC4010/BC4015 Soil β-Xylosidase(S-β-XYS) Activity Assay Kit
BC3080/BC3085 Soil α-glucosidase(S-α-GC) Activity Assay Kit
BC0240/BC0245 Soil Saccharase(S-SC) Activity Assay Kit