Soil Neutral Invertase (S-NI) Activity Assay Kit

Operation Equipment: Spectrophotometer/ Microplate reader

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

Catalog Number: BC4045

Size: 100T/48S

Components:

Reagent I: 40 mL×1. Storage at 4℃.

Reagent II: Powder×2. Storage at 4℃. Add 5mL of reagent I to fully dissolve for standby when the solution will be used. The unused reagents can be stored at 4℃ for two weeks.

Reagent III: 10 mL×1. Storage at 4℃.

Standard solution: powder×1, 10 mg of anhydrous glucose. Storage at 4℃; Add 1 mL of reagent I with filly dissolve before use to prepare 10 mg/mL glucose standard solution for standby. The reagents can be stored at 4℃ for two weeks.

Product Description

S-NI catalyzes the irreversible decomposition of sucrose into fructose and glucose under neutral conditions, and is one of the key enzymes for sucrose metabolism in soil microorganisms.

S-NI catalyzes the degradation of sucrose to produce reducing sugar, and further reacts with 3,5-dinitrosalicylic acid to form brownish red amino compound, which has characteristic light absorption at 540 nm, and the increase rate of light absorption at 540 nm in a certain range is in direct proportion to NI activity. Within a certain range the activity of S-NI is calculated by the increasing rate of light absorption.

Reagents and Equipment Required but Not Provided

Spectrophotometer/Microplate reader, centrifuge, constant temperature incubator/water-bath, transferpettor, micro glass cuvette/ 96-well plate, mortar, **toluene,** sieve (30-50 mesh) and distilled water.

Procedure

1. Sample preparation:
   Fresh soil samples are naturally air-dried or oven dried at 37℃ and passed through a 30-50 mesh sieve.
2. Determination steps and sample adding table:

a. Preheat spectrophotometer/ microplate reader more than 30 min, adjust wavelength to 540 nm and set zero with distilled water.

b. Dilute the standard solution with reagent I to 0.4, 0.3, 0.2, 0.1, 0.08, 0.06, 0.04 mg/mL of glucose standard solution.

c. Operate according to the following table:

Reagent Name (μL)	Test tube (T)	Control tube (C)	Standard tube (S)	Blank tube (B)
Soil sample (g)	0.02	0.02	-	

Reagent I (μL)	-	160	-	160
Reagent II(μL)	160	-		
Standard solution (μL)	-	-	160	
Toluene (μL)	4	4	4	4
Mix well. After react at 37°C for 1 hour, boil for about 10 minutes (close tightly to prevent water loss), and mix thoroughly after cooling in running water or ice bath (to ensure constant concentration), centrifuge at 10,000 rpm for 10 minutes at room temperature, and take the supernatant.				
supernatant	140	140	140	140
Reagent III(μL)	60	60	60	60

Mix well, boil for about 10 minutes (cover tightly to prevent water loss). After water cooling, mix well. set zero with distilled water, record the absorption value a of each tube at 540 nm, calculate $\Delta A = A_T - A_C$, $\Delta A = A_S - A_B$

Calculation of S-NI activity:

1. The regression equation determined under standard conditions is y=kx+b; x is the concentration of standard substance (mg/mL), y is the absorption value. Take ΔA into the equation to get x (mg/mL).
2. Calculation of S-NI activity:

Unit definition: one unit is defined as an enzyme activity that the amount of enzyme that catalyzes the production of 1 mg reducing sugar per day every gram soil sample at 37°C.

S-NI activity (U/mg) =x ×V ÷W ÷T=3.84×x÷Cpr

V1: the volume of sample added into the reaction system, 0.16 mL;
W: sample fresh weight, g;
T: reaction time: 1/24d.

Note

1. If Reagent III is added and there is turbidity after boiling for 10 min, it is recommended to remove the precipitate by centrifugation and take the supernatant to determine the absorbance.
2. If the absorbance value is greater than 1.3, the sample can be diluted with distilled water and measured (multiply the corresponding dilution times in the calculation formula). If the absorbance is small, you can increase the volume of the supernatant or the fresh weight of the soil sample for measurement.

Experimental Examples:

1. Take two tubes of 0.02g forest soil, add 160μL of reagent II and 4μL of toluene to the test tube, add 160μL of reagent I and 4μL of toluene to the control tube. After an accurate water bath at

37°C for 1h, boil for 10min, centrifuge and dilute the supernatant 5 times, then follow Assay step operation, Measure with 96-well plate and calculate ΔA=At-Ac=0.127-0.074=0.053, standard curve: y=3.6555x-0.1459, x=0.05441, calculate enzyme activity:

S-NI (U/g soil) =3.84×x÷W ×5（Dilute times）=3.84×0.05441÷0.02×5（Dilute times）=52.2336 U/g soil.

2. Take two tubes of 0.02g soil sample, add 160 μL of reagent II and 4μL of toluene to the test tube, add 160 μL of reagent I and 4μL of toluene to the control tube. After an accurate water bath at 37°C for 1h, boil for 10min, centrifuge and dilute the supernatant 5 times, then follow Assay step operation, Measure with 96-well plate and calculate ΔA=At-Ac=0.115-0.068=0.047, standard curve: y=3.6555x-0.1459, x=0.05277, calculate enzyme activity:

S-NI (U/g soil) =3.84×x÷W ×5（Dilute times）=3.84×0.05277÷0.02×5（Dilute times）=50.6592 U/g soil

Related Products:

BC3070/BC3075 Soil Acid Invertase(S-AI) Activity Assay Kit