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Soil Neutral Invertase (S-NI) Activity Assay Kit

Operation Equipment: Spectrophotometer

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

Catalog Number: AK0116

Size: 50T/24S

Components:

Reagent I: 100 mL×1. Storage at 4℃.

Reagent Ⅱ: Powder×1. Storage at 4℃. Add 30 mL of Reagent I to fully dissolve for standby when the solution will be used. Store unused reagents at 4℃.

Reagent Ⅲ: 30 mL×1. Storage at 4℃.

Standard solution: powder× 1, 10 mg of anhydrous glucose. Storage at 4℃; Add 1 mL of distilled water with filly dissolve before use to prepare 10 mg/mL glucose standard solution for standby.

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, centrifuge, water-bath, transferpettor, 1 mL glass cuvette, 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 more than 30 min, adjust wavelength to 540 nm and set zero with distilled water.

b. Dilute the standard solution to 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)	0.1	0.1	-	
Reagent I (μL)	-	800	-	800

Reagent Ⅱ(μL)	800	-		
Standard solution (μL)	-	-	800	
Toluene (μL)	20	20	20	20
Mix well. After react at 37C 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	700	700	700	700
Reagent Ⅲ(μL)	300	300	300	300

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 μg reducing sugar per day every gram soil sample at 37℃.

S-NI activity (U/mg) =x ×V ÷W ÷T=19.2×x÷Cpr

V1: the volume of sample added into the reaction system, 0.8 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(10000rpm， 2min) and take the supernatant to determine the absorbance.

2. If the absorbance value is greater than 1, 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. 1g forest soil, add 800μL of reagent II and 20μL of toluene to the test tube, add 800μL of reagent I and 20μL 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, calculate ΔA=At-Ac=0. 191-0.074=0. 117, standard curve: y=5. 1864x-0. 1698, x=0.055, calculate enzyme activity:

   S-NI (U/g soil) =19.2×x÷W×5（Dilute times）=19.2×0.055÷0. 1×5（Dilute times）=52.8 U/g soil.

2. Take two tubes of 0. 1g soil sample, add 800μL of reagent II and 20μL of toluene to the test tube, add 800μL of reagent I and 20μL 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,

calculate ΔA=At-Ac=0. 161-0.086=0.075, standard curve: y=5. 1864x-0. 1698, x=0.047, calculate enzyme activity:

S-NI (U/g soil) =19.2×x÷W×5（Dilute times）=19.2×0.047÷0. 1×5（Dilute times）=45. 12 U/g soil

Related Products:

AK0157/AK0156 Soil Acid Invertase(S-AI) Activity Assay Kit