Acid Invertase (AI) Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: BC3070

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. keep the 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 Reagent I with filly dissolve before use to prepare 10 mg/mL glucose standard solution for standby.

Product Description

S-AI catalyzes the irreversible decomposition of sucrose into fructose and glucose at pH 4.5 to 5.0 (acidic). It is one of the key enzymes for sucrose metabolism in soil microorganisms.

S-AI 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. 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/ homogenizer, **toluene,** sieve (50 mesh, or smaller) 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 0.2, 0.15, 0.1, 0.08, 0.06 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 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	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. zero adjustment of distilled water at 540nm. record the absorption value a of each tube at 540 nm as A_T, A_C, A_S, A_B, calculate $\Delta A = A_T - A_C$, $\Delta A = A_S - A_B$

Calculation of 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 (μmol/mL).

2. Calculation of S-NI activity:

1) Calculate by protein concentration:

Unit definition: one unit is defined as an enzyme activity that enzyme catalyzes the production of 1 μg of reducing sugar per minute at 37℃ every gram soil.

S-AI 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 value is small, the dilution times of supernatant can be reduced. Both operations should pay attention to changing the dilution ratio in the formula.

Experimental Example:

1. Take two tubes of 0.02 g forest soil, which are test tube and control tube. Operate according to the measuring steps, and record them as A_T and A_C. Calculation: $A=A_T-A_C=0.425-0.054=0.371$, The standard curve is y=6.0331x-0.3103，x=0.113

S-AI (U/g soil sample) = 19.2×x÷W=19.2×0.113÷0.1=21.696 U/g soil sample.

2. Take two tubes of 0.02g forest soil, which are measuring tube and control tube. Operate according to the measuring steps, and record them as A_T and A_C. Calculation: $A=A_T-A_C= 0.410-0.055 =0.355$, The standard

curve is y=6.0331x-0.3103，x=0.110

S-AI (U/g soil sample) = 19.2×x÷W=19.2×0.110÷0.1=21.12 U/g soil sample.

Related Products：

BC4040/BC4045 Soil Neutral Invertase(S-NI) Activity Assay Kit

BC0240/BC0245 Soil Saccharase(S-SC) Activity Assay Kit