Soil Cellulase (S-CL) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/ Microplate reader

Catalog Number: NA0403

Size:100T/48S

Components:

Reagent I: Methylbenzene, Liquid 2.5 mL×1. Storage at 4℃. Requird but not provided.

Reagent II: Liquid 5 mL×1. Storage at 4℃.

Reagent III: Liquid 20 mL×1. Storage at 4℃.

Reagent IV: Liquid 6 mL×1. Storage at 4℃.

Standard: Powder×1. Storage at 4℃. Contain 10 mg of anhydrous glucose (dry weight loss < 0.2%). Dissolve the standard with 1 mL of distilled water to generate a 10 mg/mL glucose solution standard. Store at 4℃ and use within one week or dissolve the standard with saturated benzoic acid solution for a longer time.

Product Description

Soil Cellulase (S-CL) mainly comes from soil microorganisms. Glucose produced by S-CL is the main carbon source nutrients of soil microorganisms. In this kit, this product uses the 3.5-dinitrosalicylic acid method to determine the content of reducing sugars produced by S-CL catalyzing cellulose degradation.

Reagents and Equipment Required but Not Provided.

Water-bath, transferpettor, micro glass cuvette/96 well flat-bottom plate, spectrophotometer/microplate reader, toluene (express delivery is not allowed), ice and distilled water.

Procedure:

I. Crude enzyme extraction:

Add 0.05 mL of methylbenzene to 0.1 g of dry soil sample, shake slightly for 15 minutes. Add 0.4 mL of Reagent I, mix thoroughly and keep in 37℃ incubator for 24 hours. Add 1 mL of Reagent II immediately and mix thoroughly to stop the catalysis. Centrifuge at 10000 rpm for 10 minutes at room temperature, take the supernatant on ice for testing.

II. Determination procedure:

1. Preheat the spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to 540 nm, and set zero with distilled water.

2. Standard preparation: Dilute the standard to 1, 0.8, 0.6, 0.4, 0.2, 0.1 mg/mL with distilled water.

3. Add the following reagents to the EP tube in turn:

Reagent	Contrast Tube (C)	Test Tube (T)	Standard Tube (S)	Blank Tube (B)
Water-free soil (g)	0.05	0.05	-	-

Reagent I (μL)	25	25	-	-
	Boil for 15 minutes (take the lid tightly close)	Shack to mix thoroughly, place at RT for 15 minutes.	-	-
Reagent II (μL)	45	45	-	-
Reagent III (μL)	185	185	-	-
Distilled water (μL)	45	45	-	-
Shake to mix thoroughly, then saccharification in water bath at 40℃ for 1 hour. After the saccharification, boil for 15 minutes (tightly closed to prevent moisture loss), then get saccharified liquid.				
Saccharified liquid (μL)	15	15	-	-
Standard solution (μL)	-	-	15	-
Distilled water (μL)	-	-	-	15
Reagent IV (μL)	35	35	35	35
Mix thoroughly, boil in boiling water bath for 15 minutes (cover tightly to prevent water loss), then leave the tube to cool.				
Distilled water (μL)	250	250	250	250
Mix thoroughly. After cooling, take 200 μL to micro glass cuvette or 96 well flat-bottom plate, then detect the absorbance at 540 nm and noted as A_C, A_T, A_S, andA_B. $\Delta A_T=A_T-A_C$. $\Delta A_S=A_S-A_B$. Each test tube should be provided with a contrast tube.				

Standard curve: Adjust to zero with distilled water at 540 nm. Standard tube absorption value $\Delta A_S=A_S-A_B$. It is established with concentration (y) as ordinate and absorbance ΔA_S (x) as abscissa to obtain the equation y=kx+b.

Calculation

Standard curve: Adjust to zero with distilled water at 540 nm. Standard tube absorption value $\Delta A_S=A_S-A_B$. It is established with concentration (y) as ordinate and absorbance ΔA_S (x) as abscissa to obtain the equation y=kx+b.

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 milligram of glucose in the reaction system per day every gram soil sample.

S-CL (U/g) = $y\times Vr\div W\div T = 144\times y$

T: Reaction time, 1 hour=1/24 day;

Vr: Total reaction volume, 0.3 mL;

W: Sample weight, 0.05 g.

Note:

Prolong the 40℃ water bath reaction time if the absorbance of sample tube is too small (0.02), convert the reaction time when finally calculate.

References：

[1] Deng S P, Tabatabai M A. Cellulase activity of soils[J]. Soil Biology and Biochemistry, 1994, 26(10): 1347-1354.

[2] Sinegani A A S, Sinegani M S. The effects of carbonates removal on adsorption, immobilization and activity of cellulase in a calcareous soil[J]. Geoderma, 2012, 173: 145-151.

Related Products：

NA0846/NA0604 Soil Alkaline Phosphatase(S-AKP/ALP) Activity Assay Kit
NA0862/NA0619 Soil Polyphenoloxidase (S-PPO) Activity Assay Kit
NA0618/NA0618 Soil Urease(S-UE) Activity Assay Kit
NA0860/NA0617 Soil Acid Phosphatase(S-ACP) Activity Assay Kit