SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

Soil Cellulase (S-CL) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: AK0588

Size:50T/24S

Components:

Reagent I: Methylbenzene, Liquid 10 mL×1. Storage at 4℃ . Requird but not provided.

Reagent Ⅱ: Liquid 15 mL×1. Storage at 4℃ .

Reagent Ⅲ: Liquid 50 mL×1. Storage at 4℃ .

Reagent ⅠⅤ: Liquid 15 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, spectrophotometer, 1 mL glass cuvette, methylbenzene (express delivery is not allowed), ice and distilled water.

Procedure

1. Preheat the spectrophotometer 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 reagents as the following table.

Reagent	Contrast Tube (C)	Test Tube (T)	Standard Tube (S)	Blank Tube (B)
Water-free soil (g)	0.25	0.25	-	-
Reagent I (μL)	125	125	-	-
	Boil for 15 minutes (take the lid tightly close)	Shack to mix thoroughly, place at RT for 15 minutes.	-	-
Reagent Ⅱ (μL)	250	250	-	-

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Reagent Ⅲ (μL)	1000	1000	-	-
Distilled water (μL)	250	250	-	-
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)	50	50	-	-
Standard solution (μL)	-	-	50	-
Distilled water (μL)	-	-	-	50
Reagent Ⅳ (μL)	150	150	150	150
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)	1050	1050	1050	1050
Mix thoroughly, then detect the absorbance at 540 nm and noted as A_c, A_T, A_S, and A_B. $\Delta A_T=A_T-A_C$. $\Delta A_S=A_S-A_B$.				

Standard curve: Adjust to zero with distilled water at 540 nm. Standard tube absorption value $\Delta A_S=A_S-A_B$. Contrast Tube (C) is set for each tube.

Calculation

According to the standard curve, add sample ΔA_T into the formula (x) to calculate the sample concentration y (mg/mL).

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×Vr÷W÷T =156×y

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

Vr: Total reaction volume, 1.625 mL;

W: Sample weight, 0.25 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.

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