Soil β-1,4-Glucanase / Cellobiosidase (S-C1) Activity Assay Kit

Operation Equipment: Spectrophotometer

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

Catalog Number: BC4030

Size: 50T/24S

Components:

Reagent I: Toluene 3mL×1. Storage at 4℃. **(self-provided reagent)**

Reagent II: Powder×2. Storage at 4℃. Add 10 mL of Reagent III to fully dissolve when the solution will be used. The left reagent can be stored at 4℃ for one weeks.

Reagent III: 50 mL×1. Storage at 4℃.

Reagent IV: 60 mL×1. Storage at 4℃.

Standard solution: 1mL×1, 5mmol/L p-nitrophenol solution. The standard is diluted 50 times with reagent III to obtain a 100 μmol/L standard solution.

Product Description

β-1,4-glucanase/cellobiosidase (C1, EC3.2.1.91) exists in bacteria, fungi and animals, and is a component of the cellulase system. The end of the linear molecule hydrolyzes the β-glucosidic bond and cuts out one cellobiose molecule every time.

S-C1 can catalyze p-nitrobenzene cellobiose (PNPC) to p-nitrophenol, which has a characteristic light absorption at 400nm.

Reagents and Equipment Required but Not Provided

Spectrophotometer, centrifuge, water-bath/ Constant temperature incubator, 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 400 nm and set zero with distilled water.

b. Standard dilution: Take 20μL of 5 mmol/L p-nitrophenol solution before use, add 980μL of distilled water, mix well, and make a 100 μmol/L standard solution for use, ready to use. (In the experiment, each tube needs 500μL, in order to reduce the experiment error, so prepare a large volume.)

c. Operate according to the following table:

Reagent Name	Test tube (T)	Control tube (C)	Standard tube (S)	Blank tube (B)
Soil sample (g)	0.1	0.1		
Reagent I (μL)	50	50		
Mix by shaking to make the soil sample wet and leave it for 15min at room temperature				
Reagent II (μL)	400			
Reagent III (μL)	500	500		
Mix well. After reacting for 1 h at 37℃ in a water bath, immediately boil in a water bath for 5 min (close tightly to prevent water loss) and cool in running water/ice bath.				
Reagent II (μL)		400		
Centrifuge at 10,000 rpm and 25℃ for 10 min and take the supernatant				
Supernatant	500	500		
Standard solution (μL)	-	-	500	
Distilled water (μL)				500
Reagent IV (μL)	1000	1000	1000	1000

Mix well, react for 2 minutes at RT. record the absorption value a of each tube at 400 nm, calculate $\Delta A = A_T - A_C$, $\Delta A_S = A_S - A_B$

Calculation of S-C1 activity:

1. Calculation of S-C1 activity:

Unit definition: one unit is defined as the amount of enzyme that catalyzes the production of 1 μmol of p-nitrophenol per day every gram of soil catalyzes.

S-C1 activity (U/g soil sample) $= \Delta A \div (\Delta A_S \div C_S) \times V1 \div W \div T = 2.28 \times \Delta A \div \Delta A_S \div W$

C_S: concentration of standard solution，100μmol/L

V1: the volume of reaction system, 9.5×10^{-4} L;

W: sample fresh weight, g;

T: reaction time: 1/24d.

Note

1. If the absorbance value is greater than 1.5, it is recommended to dilute the supernatant with reagent III and determine with decrease the quality of soil samples.

Experimental Examples:

1. Take two tubes of 0.1g soil sample, which are the measuring tube and the control tube. Follow the measuring steps and mark them as At and Ac. Calculate ΔAt=At-Ac=0.79-0.308=0.482, ΔAs=As-A b=0.599-0=0.599, calculate the enzyme activity:

S-C1 activity (U/g soil sample) =2.28×ΔAt÷ΔAs÷W=2.28×0.482÷ 0.599÷0.1=18.3466 U/g soil sample.

2. Take two tubes of 0.1g forest soil samples, which are the measuring tube and the control tube. Follow the measuring steps and mark them as At and Ac. Calculate ΔAt =At-Ac=0.613-0.346=0.267, ΔA s=As-Ab=0.599-0=0.599, calculate the enzyme activity:

S-C1 activity (U/g soil sample) =2.28×ΔAt÷ΔAs÷W=2.28×0.267÷0.599÷0.1=10.163U/g soil sample

Related Products:

BC4010/BC4015 Soil β-Xylosidase (S-β-XYS) Activity Assay Kit

BC3080/BC3085 Soil α-glucosidase (S-α-GC) Activity Assay Kit

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

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