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: NA0361

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. Store unused reagents at 4°C

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 100μmol/L standard solution before use.

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, 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. 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				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 at 37℃.

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

C_S：concentration of standard solution，100 μ mol/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-Ab=0.599-0=0.599, calculate the enzyme activity:
   S-C1 activity (U/g soil) ）=2.28×ΔAt÷ΔAs÷W=2.28×0.482÷ 0.599÷0.1=18.3466 U/g soil.
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, ΔAs=As-Ab=0.599-0=0.599, calculate the enzyme activity:
   S-C1 activity (U/g soil) =2.28×ΔAt÷ΔAs÷W=2.28×0.267÷0.599÷0.1=10.163U/g soil

Related Products:

NA0364/NA0363 Soil β-Xylosidase(S-β-XYS) Activity Assay Kit
NA0645/NA0404 Soil α-glucosidase(S-α-GC) Activity Assay Kit
NA0850/NA0608 Soil Saccharase(S-SC) Activity Assay Kit