Soil β-Xylosidase (S-β-XYS) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: BC4010

Size： 50T/24S

Components:

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

Reagent II: Liquid 30 mL×1. Storage at 4℃.

Reagent III: Powder×2. Storage at -20℃. Add 10 mL of distilled water to each bottle before use. The left reagent can be stored at -20℃ for four weeks.

Reagent IV: Liquid 60 mL×1. Storage at 4℃.

Standard: Liquid 1 mL×1. Storage at 4℃. 5 mmol/L p-nitrophenol solution.

Product Description:

Soil β-xylosidase (S-β-XYS) exists in organisms such as plants, bacteria and fungi, which is a key enzyme that catalyzes the degradation of xylan hemicellulose. The product xylose can be used as a carbon source in microbial fermentation. In addition, β-XYS can also be used as a biological bleaching agent in the paper industry, which is more environment-friendly than traditional bleaching methods and has a widespread application value.

S-β-XYS can catalyze the p-nitrophenyl beta-xylopyranoside to p-nitrophenol. The product has characteristic of absorption at 400 nm. In this kit, the S-β-XYS activity is quantified by measuring the increase in the color development at 400 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, water-bath, desk centrifuge, transferpettor, 1 mL glass cuvette, analytical balance, mortar, 30-50 mesh sieve, **methylbenzene**, ice and distilled water.

Procedure:

I. Preparation of samples

Fresh soil samples are naturally air-dried or oven-dried at 37℃, pass through a 30-50 mesh sieve.

II. Determination procedure:

1. Preheat Spectrophotometer for 30 minutes, adjust the wavelength to 400 nm, set zero with distilled water.
2. Dilution of standard solution: Take 20μL of 5 mmol/L p-nitrophenol solution before use, add 980μL of reagent II, 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.)
3. Add reagents in 1 mL glass cuvette as the following:

Reagent	Test tube (T)	Contrast Tube (C)	Standard tube (S)	Blank tube (B)
Air-dried soil (g)	0.1	0.1	-	-
Reagent I (μL)	20	20	-	-
The soil samples are all wetted by oscillating mixing, and store at 25℃ for 15 minutes.				
Reagent II (μL)	500	500	-	-
Reagent III (μL)	400	-	-	-
Mix thoroughly and incubate the reaction for 1 hour at 50℃ water bath, then take the reaction solution in a boiling water bath for 5 minutes immediately (tightly close to prevent moisture loss), flowing water to cool.				
Reagent III (μL)	-	400	-	-
Mix thoroughly, centrifuge at 10000 rpm for 10 minutes at 25℃ and take the supernatant.				
Supernatant (μL)	500	500	-	-
Standard solution (μL)	-	-	500	-
Distilled water (μL)	-	-	-	500
Reagent IV (μL)	1000	1000	1000	1000

Mix thoroughly and stand at room temperature for 2 minutes, centrifuge at 10000×g for 5 minutes. Take the supernatant and detect the absorbance of each tube at 400 nm and noted as A_T, A_C, A_S and A_B. Calculate $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$. Each test tube should be provided with one contrast tube.

III. S-NAG activity calculation:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the generation 1μmol of p-nitrophenol every gram of soil sample in the reaction system per day.

S-β-XYS (U/g soil sample) $=\Delta A_T \div (\Delta A_S \div C) \times Vrv \div W \div T = 2.21 \times \Delta A_T \div \Delta A_S \div W$

C: Concentration of standard solution, 100 μmol/L;

Vrv: Total volume in catalyze system, 9.2×10^{-4} L;

W: Soil sample weight, g;

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

Experimental Examples:

1. Take two tubes of 0.1g soil, which are the measuring tube and the control tube. Follow the measuring steps and mark them as At and Ac. Calculate ΔAt=At-Ac=0.46-0.221=0.239, ΔAs=As-Ab=0.568-0.002=0.566, calculate the enzyme activity:
   S-β-XYS activity (U/g soil sample)= 2.21×ΔAt÷ΔAs÷W=2.21×0.239÷0.566÷0.1=9.3312 U/g.
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=0.356-0.109=0.247, ΔAs=As-Ab=0.568-0.002=0.566, calculate enzyme activity:
   S-β-XYS activity (U/g soil sample) =2.21× ΔAt÷ΔAs÷W=2.21×0.247÷0.566÷0.1=9.6443 U/g.

Related Products:

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

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