

β - xylosidase Activity Assay Kit

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

Detection instrument: Spectrophotometer

Cat No: NA0681

Size: 50T/25S

Components:

Extract solution: 50 mL \times 1. Store at 4°C.

Reagent I: Powder \times 1. Store at -20°C. Fully dissolved with 2 mL distilled water before use.

Reagent II: 35 mL \times 1. Store at 4°C.

Reagent III: 35 mL \times 1. Store at 4°C.

Standard: 1 mL \times 1. Store at 4°C. 5 μ mol/mL p-nitrophenol solution.

Product Description:

β -xylosidase (ec3.2.1.37) exists in plants, bacteria, fungi and other organisms. It is the key enzyme to catalyze the degradation of xylanase hemicellulose. Xylose can be used as a carbon source for microbial fermentation. In addition, β -xylosidase can also be used as a biological bleaching agent in papermaking industry, which is more environmentally friendly than the traditional bleaching method and has a wide range of application value.

β -xylosidase catalyzes p-nitrophenol- β -D-xyloside to produce p-nitrophenol. P-nitrophenol has a characteristic absorption peak at 405 nm. The increase rate of light absorption at 405 nm can be measured to calculate the activity of β -xylosidase.

Required material

Scales, low temperature centrifuge, spectrophotometer, 1 mL glass cuvette, mortar/homogenizer, water bath and distilled water.

Procedure:

I. Extract of crude enzyme:

1. Tissue sample:

Weigh about 0.1 g of the sample, then add 1.0 mL of the Extract solution, fully homogenate in the ice bath, then centrifuge at 12000 rpm for 20 minutes at 4°C. Discard the precipitate, take 20 μ L of the supernatant to determine the protein content, and the remaining supernatant as the enzyme solution to be tested.

2. Bacteria or cells:

Collect about 5 million cells, add 1.0 mL of Extract solution, break cells with ultrasonic (ice bath, power 300W, ultrasonic for 3 seconds, interval 7 seconds, the total time of 3 minutes). Centrifuge at 10000 rpm for 10 minutes at 4°C. Discard the sediment, take 20 μ L of supernatant to determine the protein content, and put the remaining supernatant on the ice for testing.

II. Determination procedure:

1. Preheat the spectrophotometer for 30 minutes, adjust wavelength to 405 nm, set zero with distilled water.

2. Standard working solution:

Dilute the standard solution to 0.2, 0.1, 0.05, 0.025, 0.0125, 0.00625 $\mu\text{mol/mL}$ with Reagent II.

3. Add reagents with the following list:

Reagent name (μL)	Control tube (C)	Test tube (T)	Blank tube (B)	Standard tube (S)
Crude enzyme solution	200	200	-	
Standard	-	-	-	200
Reagent I	-	50	-	
Reagent II	400	350	600	400
Mix well, 45°C water bath for 20 minutes .				
Reagent III	400	400	400	400
Mix well, let it stand for 5 minutes, 1 mL glass cuvette, set zero with distilled water, measure the light absorption value A_{405} of 405 nm, record as A_C , A_T , A_B and A_S respectively. And calculate $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$.				

III. Calculation formula of β -xylosidase activity:

1. Create standard curve

A standard curve was established based on the concentration(y) of the standard tube and the absorbance $\Delta A_S(x)$. According to the standard curve, calculate amount of product generated by sample ($\mu\text{mol/mL}$) by taking $\Delta A_T(x)$ into the formula.

1) Calculated by protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 μmol of p-nitrophenol in the reaction system per minute at 45°C and pH 7.4 every mg protein.

$$\beta\text{-xylosidase } (\mu\text{mol}/\text{min}/\text{mg prot}) = (y \times V_S) \div (V_S \times C_{pr}) \div T = 0.05 \times y \div C_{pr}$$

2) Calculated by sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 μmol of p-nitrophenol in the reaction system per minute at 45°C and pH 7.4 every g sample.

$$\beta\text{-xylosidase } (\mu\text{mol}/\text{min} / \text{g flash weight}) = (y \times V_S) \div (W \times V_S \div V_{ST}) \div T = 0.05 \times y \div W$$

3) Calculated by bacteria or cell amount:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 μmol of p-nitrophenol in the reaction system per minute at 45°C and pH 7.4 every 10^4 bacteria or cells.

$$\beta\text{-xylosidase } (\mu\text{mol}/\text{min} / 10^4 \text{ cell}) = (y \times V_S) \div (500 \times V_S \div V_{ST}) \div T = 0.0001 \times y$$

V_S : The volume of sample added to the reaction system, 0.2 mL;

V_{ST} : The total volume of added extract, 1 mL;

C_{pr} : Supernatant protein concentration, mg/mL;

W: Sample weight, g;

500: Bacteria/cell amount, 5 million;

T: Reaction time, 20 minutes;

Note:

1. The absorbance should be controlled between 0.05 and 0.6. Otherwise, increase the sample quantity or dilute the sample, and pay attention to the change of the dilution ratio involved in the calculation formula.
2. The protein content of the sample needs to be determined separately, and the Coomassie brilliant blue method protein content determination kit can be selected for determination.

Related Products:

NA0838/NA0596 β -1,3-glucanase(β -1,3-GA) Activity Assay Kit

NA0687/NA0446 α -glucosidase(α -GC) Activity Assay Kit

NA0686/NA0445 β -glucosidase(β -GC) Activity Assay Kit

NA0685/NA0444 α -galactosidase(α -GAL) Activity Assay Kit