

β -xylosidase Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Detection instrument: Spectrophotometer/Microplate reader

Cat No: BC2625

Size: 100T/48S

Components:

Extract: Liquid 100 mL \times 1. Store at 4°C.

Reagent I: Powder \times 2. Store at -20°C. Before use, take 1 tube and add 0.555 ml distilled water to fully dissolve it. The unused reagent can be stored at -20°C for 2 weeks. Avoid repeated freezing and thawing.

Reagent II: Liquid 15 mL \times 1. Store at 4°C.

Reagent III: Liquid 15 mL \times 1. Store at 4°C.

Standard: Liquid 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/microplate reader, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, water bath and distilled water, ultrasonic crusher.

Procedure:

I. Extract of crude enzyme:

1. Tissue sample:

Weigh about 0.1 g of the sample, then add 1 mL of the Extract solution, 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 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), then 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/microplate reader for 30 minutes, adjust wavelength to 405 nm. The spectrophotometer needs to be zeroed 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	40	40	-	-
Standard	-	-	-	40
Reagent I	-	10	-	-
Reagent II	80	70	120	80
Mix well, 45°C water bath for 20 minutes.				
Reagent III	80	80	80	80

Mix well, let it stand for 5 minutes, measure the light absorption value of 405 nm with micro glass cuvette /96 well plate, record as A_C , A_T , A_B and A_S respectively. And calculate $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$. Each Test tube shall be provided with a control tube. The standard tube and blank tube only need to be measured 1-2 times.

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.

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

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.

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

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.

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

V_s : The volume of sample added to the reaction system, 0.04 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:

BC0360/BC0365 β -1,3-glucanase(β -1,3-GA) Activity Assay Kit

BC2550/BC2555 α -glucosidase(α -GC) Activity Assay Kit

BC2560/BC2565 β -glucosidase(β -GC) Activity Assay Kit

BC2570/BC2575 α -galactosidase(α -GAL) Activity Assay Kit