β- xylosidase Activity Assay Kit

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

Detection instrument: Spectrophotometer/Microplate reader

Cat No: NA0439

Size: 100T/48S

Components:

Extract: 100 mL×1. Store at 4℃.

Reagent I: Powder×1. Store at -20℃. Fully dissolved with 1 mL of distilled water before use.

Reagent II: 15 mL×1. Store at 4℃.

Reagent III: 15 mL×1. Store at 4℃.

Standard: Liquid×1. Store at 4℃. 5 μmol/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.

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℃. Discard the precipitate, take 20 μL 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℃. Discard the sediment, take 20 μL 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, set zero with diatilled water.

2. Standard working solution:

Dilute the standard solution to 0.2, 0.1, 0.05, 0.025, 0.0125, 0.00625 μmol/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℃ 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 $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$.				

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 (μmol/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℃ and pH 7.4 every mg protein.

β-xylosidase (μmol/min/mg prot)=$(y \times V_S) \div (V_S \times Cpr) \div T = 0.05 \times y \div Cpr$

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℃ and pH 7.4 every g sample.

β-xylosidase (μmol/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℃ and pH 7.4 every 10^4 bacteria or cells.

β-xylosidase (μmol/min /10^4 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;

Cpr: 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