# β- xylosidase Activity Assay Kit

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

**Detection instrument:** Spectrophotometer

**Cat No:** BC2620

**Size:** 50T/24S

**Components:**

**Extract solution**: Liquid 50 mL×1. Store at 2-8°C.

**Reagent I**: Powder×2. Store at -20°C. Before use, take one Reagent I and add 1 mL distilled water to fully dissolve it. The unused reagent can be stored at -20°C for 2 weeks after thawing to avoid repeated freezing and thawing.

**Reagent II**: Liquid 35 mL×1. Store at 2-8°C.

**Reagent III**: Liquid 35 mL×1. Store at 2-8°C.

**Standard**: Liquid 1 mL×1. Store at 2-8°C. 5 μmol/mL p-nitrophenol solution.

**Product Description:**

β-xylosidase (EC 3.2.1.37) 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.

**Reagents and Equipment Required but Not Provided:**

Balance, low temperature centrifuge, spectrophotometer, 1 mL glass cuvette, mortar/homogenizer/cell ultrasonic crusher, 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 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 1.0 mL of Extract solution, break cells with ultrasonic (ice bath, power 200W, 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 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 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 (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℃ water bath for 20 minutes . | | | | |
| Reagent III | 400 | 400 | 400 | 400 |
| Mix well, let it stand for 5 minutes, measure the light absorption value of 405 nm, record as $A_C$, $A_T$, $A_B$ and $A_S$ respectively. And calculate $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$. Each Test tube shall be provided with a control tube. The standard curve 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 (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 activity (U/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 activity (U/g 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 activity (U/$10^4$ 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, 0.2 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:**

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|---|---|
| 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 |