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CheKine™ Mirco β-Xylosidase (β-X) Activity Assay Kit

Cat #: KTB1329 Size: 48 T/96 T

	Mirco β-Xylosidase (β-X) Activity Assay Kit		
REF	**Cat #:** KTB1329	LOT	**Lot #:** Refer to product label
	Applicable sample: Plant tissues, Bacteria, and Fungi		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

β-Xylosidase (EC 3.2.1.37) exists in organisms such as plants, bacteria, and fungi. It is a key enzyme that catalyzes the degradation of xylan-based hemicelluloses, and the product xylose can serve as a carbon source for microbial fermentation. Additionally, β-xylosidase can be used as a biobleaching agent in the paper industry, offering environmental benefits over traditional bleaching methods and possessing broad application value. CheKine™ Mirco β-Xylosidase (β-X) Activity Assay Kit offers a simple, convenient, and rapid approach for assessing β-Xylosidase activity, which is suitable for plant tissue, bacteria, and fungi samples. The principle involves β-xylosidase catalyzing the production of p-nitrophenol from p-nitrophenyl-β-D-xyloside. p-Nitrophenol exhibits a characteristic absorption peak at 405 nm. By measuring the rate of increase in absorbance at 405 nm, the β-xylosidase activity can be calculated.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent I	50 mL	100 mL	4℃
Reagent II	0.5 mL	1 mL	4℃, protected from light
Reagent III	5 mL	10 mL	4℃
Reagent IV	5 mL	10 mL	4℃
Standard	1 mL	1 mL	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 405 nm
- Water bath, analytical balance, ice maker, low-temperature centrifuge
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Deionized water

• Dounce homogenizer

Reagent Preparation

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent II: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent IV: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Standard: Ready to use as supplied, a 5 μmol/mL p-nitrophenol standard solution. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Standard preparation: Using 5 μmol/mL p-nitrophenol standard solution, prepare standard curve dilution as described in the table:

Num.	Standard Volume	Reagent III Volume (μL)	Concentration (μmol/mL)
Std.1	40 μL 5 μmol/mL Standard	460	0.4
Std.2	200 μL of Std.1 (0.4 μmol/mL)	200	0.2
Std.3	200 μL of Std.2 (0.2 μmol/mL)	200	0.1
Std.4	200 μL of Std.3 (0.1 μmol/mL)	200	0.05
Std.5	200 μL of Std.4 (0.05 μmol/mL)	200	0.025
Std.6	200 μL of Std.5 (0.025 μmol/mL)	200	0.0125
Std.7	200 μL of Std.6 (0.0125 μmol/mL)	200	0.0063

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. For plant tissues: Homogenize the tissue in an ice bath at a ratio of tissue mass (g): Reagent Ⅰ volume (mL) of 1:5~10 (it is recommended to weigh approximately 0.1 g of plant tissue and add 1 mL of Reagent Ⅰ). Then centrifuge at 10,000 g and 4℃ for 20 min, and retain the supernatant for analysis.
2. For bacteria and fungi: Break the cells using an ice bath and sonication at a ratio of bacterial or fungal count. Use sonication at 300 W power, sonicating for 3 s and pausing for 7 s, for a total time of 3 min. Then centrifuge at 10,000 g and 4℃ for 10 min, and retain the supernatant on ice for analysis.

Note: 1. The sample must be kept on ice throughout the entire process, otherwise, the activity will decrease. Conduct the assay as soon as possible after sample extraction.

2. If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 405 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Operation table (The following operations are operated in a 96-well plate or microglass cuvette):

Reagent	Control Well (μL)	Test Well (μL)	Standard Well (μL)	Blank Well (μL)
Supernatant	40	40	0	0

Standard	0	0	40	0
Reagent II	0	10	0	0
Reagent III	80	70	80	120
Mix well and incubate in a 45℃ water bath for 20 min				
Reagent IV	80	80	80	80
Mix well, let stand at room temperature for 10 min, and measure the absorbance at 405 nm, recording the values as $A_{Control}$, A_{Test}, $A_{Standard}$ and A_{Blank}. Calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.				

Note: Before the experiment, it is suggested that 2-3 samples with large expected differences should be selected for pre-experiment. If ΔA_{Test} is less than 0.01, it is advisable to reduce the dilution ratio or increase the sample volume appropriately. If ΔA_{Test} is greater than 0.8, the sample can be appropriately diluted with Reagent I the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve:

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve, get the standard equation y=kx+b, and bring the ΔA_{Test} into the equation to get the x value (μmol/mL).

2. Calculation of β-Xylosidase activity:

(1) Calculated by protein concentration

Active unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 nmol of p-nitrophenol per mg of protein per min at 45℃ and pH 7.4.

β-X (U/mg prot)=$(x\times V_{Sample})\div(Cpr\times V_{Sample})\div T\times 1{,}000$**$=50\times x\div Cpr$**

(2) Calculated by sample fresh weight

Active unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 nmol of p-nitrophenol per g of sample per min at 45℃ and pH 7.4.

β-X (U/g fresh weight)=$(x\times V_{Sample})\div(W\times V_{Sample}\div V_{Total\ Sample})\div T\times 1{,}000$**$=50\times x\div W$**

(3) Calculated by number of bacteria or fungi

Active unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 nmol of p-nitrophenol per 10^4 of bacteria or fungi per min at 45℃ and pH 7.4.

β-X ($U/10^4$)=$(x\times V_{Sample})\div(N\times V_{Sample}\div V_{Total\ Sample})\div T\times 1{,}000$**$=50\times x\div N$**

Where: Cpr: sample protein concentration, mg/mL; V_{Sample}: sample volume added, 0.04 mL; $V_{Total\ Sample}$: Reagent I volume added, 1 mL; W: Sample fresh weight, g; N: Total number of bacteria or fungi, 10^4; T: Reaction time, 20 min; 1,000: 1 μmol/mL=1,000 nmol/mL.

Typical Data

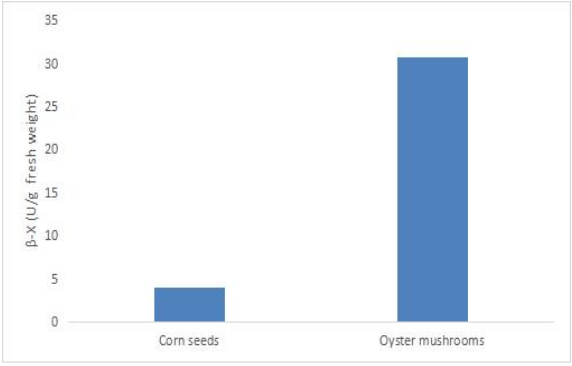

Figure 1. Determination β-Xylosidase activity in Corn seeds and Oyster mushrooms by this assay kit

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-Glucosidase Activity Assay Kit
KTB1121	CheKine™ Pyruvate Acid (PA) Colorimetric Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.