CheKine™ MircoSoil β-Xylosidase (S-β-XYS) Activity Assay Kit

Cat #: KTB4025 Size: 48 T/48 S 96 T/96 S

	Mirco Soil β-Xylosidase (S-β-XYS) Activity Assay Kit		
REF	**Cat #:** KTB4025	LOT	**Lot #:** Refer to product label
	Applicable sample: Soil		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Soil β-Xylosidase (S-β-XYS) 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 Soil β-Xylosidase (S-β-XYS) Activity Assay Kit offers a simple, convenient, and rapid approach for assessing soil β-Xylosidase activity. 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	Powder×1 vial	Powder×2 vials	-20℃, protected from light
Reagent Ⅱ	15 mL	30 mL	4℃
Reagent Ⅲ	25 mL	50 mL	4℃
Standard	1 mL	1 mL	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

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, 30-50 mesh sieve
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Deionized water, toluene

Reagent Preparation

Reagent Ⅰ: Prepared before use. Add 5 mL of deionized water to each bottle, dissolve thoroughly before use, the unused Reagent Ⅰ should be packaged and stored at -20℃ for 1 month, protected from light.

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

Reagent Ⅲ: 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.

0.1 μmol/mL Standard: Prepared before use, take 20 μL of Standard, add 980 μL of Reagent Ⅱ, mix thoroughly, and obtain 0.1 μmol/mL Standard.

Note: 0.1 μmol/mL Standard needs to be prepared for each experiment, and the diluted 0.1 μmol/mL Standard should be used up within 4 h.

Sample Preparation

Note: It is recommended to use fresh soil samples.

Fresh soil samples naturally air dried or air dried in an oven at 37℃ and sieved through 30-50 mesh sieve.

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 each 1.5 mL EP tube):

Reagent	TestTube	Control Tube	Standard Tube	Blank Tube
Sample (g)	0.02	0.02	0	0
Toluene (μL)	10	10	0	0
	Shake and mix at room temperature for 15 min	Shake and mix at 90℃ for 15 min	0	0
Reagent Ⅰ (μL)	80	0	0	0
Deionized Water (μL)	0	80	0	0
Reagent Ⅱ (μL)	100	100	0	0
Mix well, incubate in a 45℃ water bath for 1 h, boil in a boiling water bath for 5 min (cover tightly to prevent water evaporation), then cool to room temperature immediately. Centrifuge 10,000 g at 25℃ for 10 min and take the supernatant for use			0	0
Supernatant	100	100	0	0
0.1 μmol/mL Standard (μL)	0	0	100	0
Deionized Water (μL)	0	0	0	100
Reagent Ⅲ	200	200	200	200
Mix well, let stand at room temperature for 2 min, centrifuge 10,000 g at 25℃ for 5 min and take 200 μL supernatant to a 96-well plate or microglass cuvette and measure the absorbance at 405 nm, recording the values as A_{Test}, $A_{Control}$, $A_{Standard}$ and A_{Blank}. Calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.				

Note: Standard Tube and Blank Tube only need to be tested 1-2 times, and each Test Tube needs to be set up a Control

Tube. 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 increase the sample weight appropriately. If ΔA_{Test} is greater than 0.8, the supernatant can be appropriately diluted with deionized water 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. Calculation of β-Xylosidase activity:

Active unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 μmol of p-nitrophenol per g of soil sample per d.

S-β-XYS (U/g Soil)= $\Delta A_{Test} \div A_{Standard} \times C_{Standard} \times V_{Total\ reaction} \div W \div T$**$=0.456 \times \Delta A_{Test} \div A_{Standard} \div W$**

Where: $C_{Standard}$: Standard concentration, 0.1μmol/mL ; $V_{Total\ reaction}$:Total volume of reaction system, 0.19 mL; W: Sample weight, g; T: Reaction time, 1 h=1/24 d.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

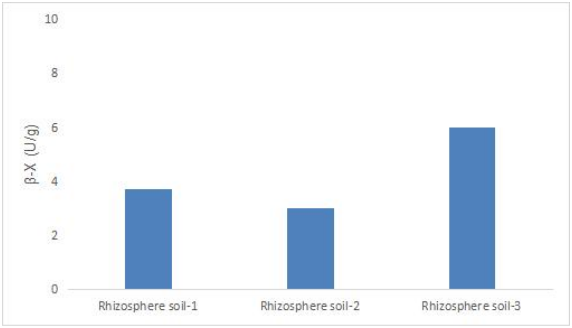

Figure 1. Determination β-Xylosidase activity in rhizosphere soil samples by this assay kit

Recommended Products

Catalog No.	Product Name
KTB4019	CheKine™ Mirco Soil α-Glucosidase (S-α-GC) Activity Assay Kit
KTB4022	CheKine™ Mirco Soil β-Glucosidase (S-β- GC) Activity Assay Kit

Disclaimer

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