CheKine™ Micro Soil Alkaline Xylanase (S-BAX) Activity Assay Kit

Cat #: KTB4045 Size: 48 T/48 S 96 T/96 S

	Micro Soil Fluorescein Diacetate (S-BAX) Activity Assay Kit		
REF	**Cat #:** KTB4045	LOT	**Lot #:** Refer to product label
	Detection range: 0.4-3 μmol/mL (1.5-11.25 U/g Soil)		**Sensitivity:** 0.4 μmol/mL (1.5 U/g Soil)
	Applicable sample: Soli		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Xylanase (EC3.2.1.8) is mainly produced by microorganisms, which can catalyze the hydrolysis of xylan, also known as pentosanase or hemicellulase, which can decompose the cell wall and β -glucan of raw materials in brewing or feed industry, reduce the viscosity of materials in brewing, promote the release of effective substances, reduce non-starch polysaccharides in feed and promote the absorption and utilization of nutrients, so it is widely used in brewing and feed industry, and BAX is generally isolated from the optimal growth. CheKine™ Micro Soil Alkaline Xylanase (S-BAX) Activity Assay Kit can detect biological samples such as soli. In this kit, S-BAX catalyzes the degradation of xylan into reducing oligosaccharides and monosaccharides in alkaline environment, and further reacts with 3,5- dinitrosalicylic acid in boiling water bath, with a characteristic absorption peak at 540 nm. The color of the reaction solution is proportional to the amount of reducing sugar produced by enzymolysis. By measuring the increase rate of absorption value of the reaction solution at 540 nm, the activity of S-BAX can be calculated.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	25 mL	50 mL	4℃
Reagent Ⅱ	4.5 mL	9 mL	4℃, protected from light
Reagent Ⅲ	20 mL	40 mL	4℃, protected from light
Standard	Powder×1 vial	Powder×1 vial	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 540 nm
- 96-well microplate or microglass cuvette, precision pipettes, disposable pipette tips,1.5 mL EP tube
- Water bath, centrifuge, 30-50 mesh sieve

• Deionized water

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℃, protected from light.

Note: Reagent III has certain irritation, so personal protection is recommended during use.

Standard: Prepared before use. Add 1 mL deionized water to a bottle, dissolve thoroughly, that is 100 μmol/mL D-Xylose Standard. The remaining reagent can also be stored at 4℃ and protected from light for 1 month.

10 μmol/mL D-Xylose Standard: Prepare 10 μmol/mL D-Xylose Standard by diluting 120 μL 100 μmol/mL D-Xylose Standard into1,080 μL deionized water. Using 10 μmol/mL D-Xylose Standard, prepare standard curve dilution as described in the table:

Num.	Standard Volume (μL)	Deionized Water (μL)	Concentration (μmol/mL)
Std.1	300 μL of 10 μmol/mL Standard	700	3
Std.2	200 μL of 10 μmol/mL Standard	800	2
Std.3	150 μL of 10 μmol/mL Standard	850	1.5
Std.4	120 μL of 10 μmol/mL Standard	880	1.2
Std.5	100 μL of 10 μmol/mL Standard	900	1
Std.6	80 μL of 10 μmol/mL Standard	920	0.8
Std.7	40 μL of 10 μmol/mL Standard	960	0.4

Note: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: 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 540 nm, visible spectrophotometer was returned to zero with deionized water.

2. Operation table (The following operations are operated in the 1.5 mL EP tube):

Reagent	Test Tube	Control Tube	Standard Tube	Blank Tube
Sample（g）	0.03	0.03	0	0
Reagent Ⅰ（μL）	150	225	0	0
Reagent Ⅱ（μL）	75	0	0	0
Mix well, incubating at 50℃ for 2 h, immediately inactivating at 90℃ for 10 min. Centrifuge at 8,000 g for 10 min at room temperature, take the supernatant, and following operations are operated in the new 1.5 mL EP tube:			0	0
Supernatant (μL)	120	120	0	0
Standard (μL)	0	0	120	0

Deionized Water (μL)	0	0	0	120
Reagent Ⅲ (μL)	120	120	120	120

Mix well, develop color at 90℃ for 5 min, cooling to room temperature with running water, take 200 μL into 96-well microplate or microglass cuvette, record the absorbance value at 540 nm. The Blank Well is recorded as A_{Blank}, the Standard Well is marked as $A_{Standard}$, the Control Well is marked as $A_{Control}$, and the Test Well is marked as A_{Test}. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: The Standard Well and Blank Well only need to be done once or twice, Each Test Well needs to be provided with a Control Well. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.01, increase the sample quantity appropriately. If ΔA_{Test} is larger than $\Delta A_{Standard}$ of 3 μmol/mL, 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. 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 and obtain the standard equation. The determination of ΔA_{Test} is brought into the equation to get x (μmol/mL).

2. Calculation of the S-BAX activity

Active unit definition: At 50℃ and pH 9.0, the amount of enzyme needed to decompose xylan to produce 1 μmol of reducing sugar per gram of soil per hour was defined as one unit of enzyme activity.

S-BAX (U/g soli)=$x\times V_{Reaction}\div W\div T\times F$**$=0.1125x\div W\times F$**

$V_{Reaction}$: Enzymatic reaction volume, 0.225 mL; T: reaction time, 2 h; W: weight of sample, g; F: dilution multiple.

Typical Data

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

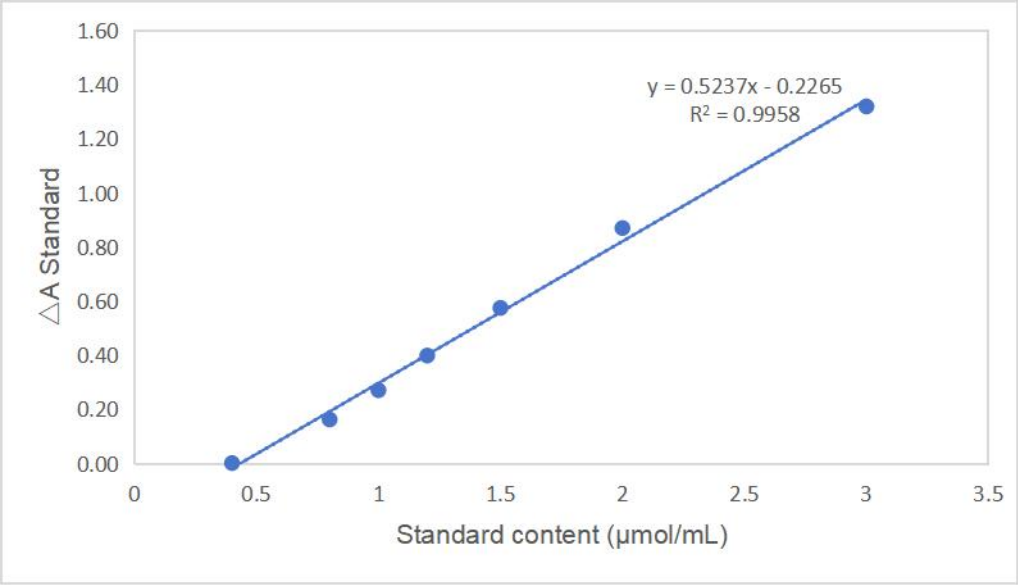

Figure 1. Standard curve of S-BAX activity.

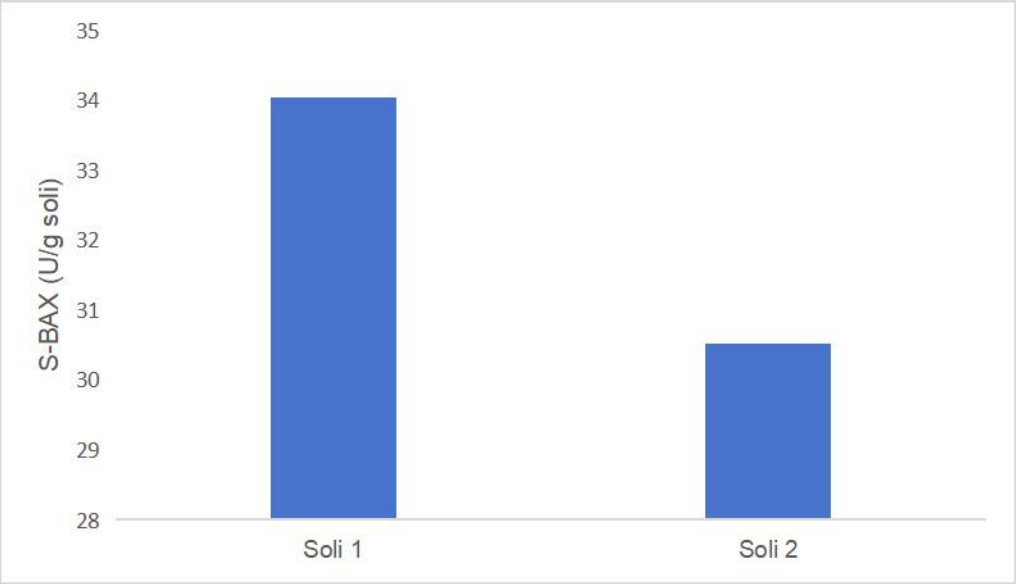

Figure 2. Determination S-BAX activity in soli sample by this assay kit.

Recommended Products

Catalog No.	Product Name
KTB4012	CheKine™ Micro Soil Nitrate Nitrogen Assay Kit
KTB4014	CheKine™ Micro Acid Soil Available Phosphorous Assay Kit
KTB4041	CheKine™ Micro Soil Alkaline Phosphatase(S-AKP/ALP) Activity Assay Kit
KTB4050	CheKine™ Micro Soil Catalase (S-CAT) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.