CheKine™ Micro Soil Neutral Xylanase (S-NEX) Activity Assay Kit

Cat #: KTB4052 Size: 48 T/48 S 96 T/96 S

	Micro Soil Neutral Xylanase (S-NEX) Activity Assay Kit		
REF	**Cat #:** KTB4052	LOT	**Lot #:** Refer to product label
	Applicable sample: Soil		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Xylanase (EC 3.2.1.8) is primarily produced by microorganisms and can catalyze the hydrolysis of xylan, also known as pentosanase or hemicellulase. It breaks down the cell walls of raw materials in brewing or feed industries and degrades β-glucans, reducing the viscosity of materials during brewing, promoting the release of effective substances, and decreasing non-starch polysaccharides in feed to enhance nutrient absorption. Therefore, it is widely used in brewing and feed industries. NEX (Neutral Xylanase) is generally isolated from microorganisms with an optimal growth pH range of 6-8. CheKine™ Micro Soil Neutral Xylanase (S-NEX) Activity Assay Kit provides a simple, convenient, and rapid method for detecting neutral xylanase activity in soil samples. The principle of this assay is that NEX catalyzes the degradation of xylan into reducing oligosaccharides and monosaccharides under neutral conditions. These products further react with 3,5-dinitrosalicylic acid (DNS) in a boiling water bath, resulting in a colorimetric reaction with a characteristic absorption peak at 540 nm. The intensity of the reaction mixture's color is proportional to the amount of reducing sugars produced by enzymatic hydrolysis. By measuring the increase in absorbance at 540 nm, the activity of S-NEX can be calculated.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	18 mL	36 mL	4℃
Reagent Ⅱ	3 mL	6 mL	4℃, protected from light
Reagent Ⅲ	13 mL	26 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: Take 120 μL of the 100 μmol/mL D-xylose standard and add 1,080 μL of deionized water to prepare a 10 μmol/mL D-xylose standard solution. Using the 10 μmol/mL D-xylose standard solution, further dilute the standard according to the table below:

Num.	Standard Volume (μL)	Deionized Water (μL)	Concentration (μmol/mL)
Std.1	200 μL of 10 μmol/mL Standard	800	2
Std.2	150 μL of 10 μmol/mL Standard	850	1.5
Std.3	120 μL of 10 μmol/mL Standard	880	1.2
Std.4	100 μL of 10 μmol/mL Standard	900	1
Std.5	80 μL of 10 μmol/mL Standard	920	0.8
Std.6	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）	0.02	0.02	0	0
Reagent Ⅰ（μL）	100	150	0	0
Reagent Ⅱ（μL）	50	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:				
Supernatant (μL)	100	100	0	0
Standard (μL)	0	0	100	0
Deionized Water (μL)	0	0	0	100

Reagent III (μL)	100	100	100	100

Mix well, incubate at 90℃ for 5 min, cooling to room temperature with running water, take 180 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 0.05, increase the sample quantity appropriately. If ΔA_{Test} is larger than $\Delta A_{Standard}$ of 2 μmol/mL, the supernatant can be further diluted with deionized water, 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-NEX activity

Active unit definition: At 50℃ and pH 6.0, 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-NEX (U/g soli)=$x\times V_{Reaction}\div W\div T\times F$**$=0.075\times x\div W\times F$**

$V_{Reaction}$: Enzymatic reaction volume, 0.15 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.

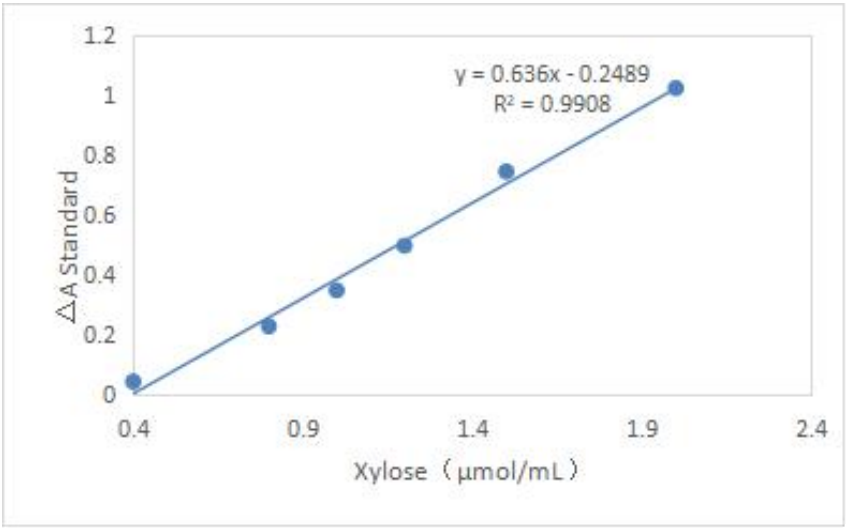

Figure 1. Standard curve of S-NEX activity.

Examples:

Take 0.02 g of fresh soil sample that has been dried in a 37℃ oven and follow the steps described above, and after an 8-fold dilution, use 96-well plate to calculate ΔA_{Test}=0.273-0.082=0.191, x=0.692. The content calculated according to the soil sample mass is as follows:

S-NEX (U/g soli)=0.075×0.692÷0.02×8=20.76 U/g.

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.