CheKine™ Micro Cellulase (CL) Activity Assay Kit

Cat #: KTB1721 Size: 48 T/24 S 96 T/48 S

	Micro Cellulase (CL) Activity Assay Kit		
REF	**Cat #:** KTB1721	LOT	**Lot #:** Refer to product label
	Applicable samples: Soil and rotten/senescent plants		
	Storage: Stored at 4℃ for 24 months, protected from light		

Assay Principle

Cellulase (EC 3.2.1.4) is a multi-enzyme system produced by microorganisms (e.g., actinomycetes, bacteria, fungi) and some animals, which acts synergistically to degrade cellulose polymers into reducing sugars. Cellulase activity measurement is important in various research fields, especially in environmental microbiology, soil ecology, plant pathology, and bioenergy development. For example, in humus soil, cellulase activity reflects the degree of organic matter decomposition and microbial metabolic activity; in senescent or rotten plant tissues, cellulase activity is often significantly high, serving as a key indicator of tissue aging, pathogen infection, and degradation.

This kit is based on the principle that cellulase catalyzes the hydrolysis of substrate to release reducing sugars such as glucose, cellobiose, and cello-oligosaccharides. The reducing sugars convert the Cu (Ⅱ)-neocuproine complex into a bright orange Cu (Ⅰ)-neocuproine complex. Cellulase activity is determined by measuring the absorbance at 450 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	60 mL	120 mL	4℃
Reagent Ⅱ	6 mL	12 mL	4℃, protected from light
Reagent Ⅲ	4 mL	8 mL	4℃, protected from light
Reagent Ⅳ	5 mL	10 mL	4℃, protected from light
Reagent Ⅴ	4 mL	8 mL	4℃
Standard	2 mL	2 mL	4℃

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 capable of measuring absorbance at 450 nm

·96-well plate, precision pipettes, disposable pipette tips, EP tubes/screw-cap tubes

·Incubator, refrigerated centrifuge, water bath/metal bath

·Deionized water

·Homogenizer, 30–50 mesh sieve (for soil samples)

Reagent Preparation

Reagent Ⅰ: Ready to use as supplied. Placed on ice during the experiment. Store at 4℃.

Reagent Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. The reagent is slightly viscous, pipette slowly to ensure accurate volume. 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.

Reagent Ⅳ: Ready to use as supplied. Equilibrate to room temperature before use. The reagent is volatile, seal tightly after each use. Store at 4℃, protected from light.

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

Standard: Ready to use as supplied. Glucose standard, concentration 2 mmol/L. Store at 4℃

Standard preparation:

Prepare standard curve dilution as described in the table:

Num.	Standard (μL)	Deionized Water (μL)	Concentration (mmol/L)
Std.1	200 μL of 2 mmol/L	200	1
Std.2	200 μL of Std.1 (1 mmol/L)	200	0.5
Std.3	200 μL of Std.2 (0.5 mmol/L)	200	0.25
Std.4	200 μL of Std.3 (0.25 mmol/L)	200	0.125
Std.5	200 μL of Std.4 (0.125 mmol/L)	200	0.0625
Std.6	200 μL of Std.5 (0.0625 mmol/L)	200	0.03125
Std.7	200 μL of Std.6 (0.03125 mmol/L)	200	0.015625
Blank	0	200	0

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, store the aliquoted soil samples at -80℃ for one month. When ready to perform the assay, thaw samples on ice. However, note that this may affect sample stability and results may be lower than expected.

1. Soil samples: Air-dry fresh soil samples or dry in 37℃ incubator, pass through a 30-50 mesh sieve. Weigh 0.1 g soil, add 0.9 mL Reagent Ⅰ, and homogenize on ice. Centrifuge at 12,000 g for 10 min at 4℃. Collect the supernatant and place it on ice to be tested.
2. Rotten/senescent plant samples: Weigh 0.1 g tissue, add 0.9 mL Reagent Ⅰ, and homogenize on ice. Centrifuge at 12,000 g for 10 min at 4℃. Collect the supernatant and place it on ice to be tested.

Assay Procedure

1. Preheat the microplate reader for more than 30 min, and adjust the wavelength to 450 nm.
2. Working Reagent Preparation: Prepare immediately before use. For each well, prepare 150 μL Working Reagent by mixing 50 μL Reagent Ⅲ, 50 μL Reagent Ⅳ, and 50 μL Reagent Ⅴ (mix in this order: first mix Reagent Ⅲ and Reagent Ⅳ, then add Reagent Ⅴ). Prepare fresh and protect from light. Don't change the order of addition.
3. Sample measurement. (Operate in EP tubes/screw-cap tubes as follows)

Reagent	Blank Tube (μL)	Standard Tube (μL)	Test Tube (μL)	Control Tube (μL)
Reagent Ⅰ	0	0	0	160
Reagent Ⅱ	0	0	160	0
Sample	0	0	40	40
Mix well, incubate at 50℃ for 1 h. After incubation, boil the tubes in boiling water for 15 min, cool to room temperature by running water, then centrifuge at 12,000 g for 10 min at room temperature. Collect the supernatant (saccharification solution) and mix well. Perform the following steps in a 96-well plate:				
Standard	0	100	0	0
Deionized Water	100	0	0	0
Saccharification solution	0	0	100	100
Working Reagent	150	150	150	150

4. Mix well, incubate at 37℃ for 40 min. The absorbance value is measured at 450 nm. Record as A_{Blank}, $A_{Standard}$, A_{Test}, $A_{Control}$. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: The saccharification solution is slightly viscous. Before pipetting into the 96-well plate, wet the tip and add slowly to ensure accurate volume. Boiling water bath can be replaced by a metal bath, make sure the temperature reaches 100℃.

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 y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve.

Bring the ΔA_{Test} of the sample into the equation to get the y value.

2. Calculation of cellulase activity:

Unit definition: One enzyme activity unit is defined as the amount of enzyme that produces 1 μg of glucose per minute per g of sample.

Cellulase activity(U/g)=$(y\times V_{Total}\times 180.16)\div(W\times V_{Sample}\div V_{Total\ sample})\div T$**$=y\times 13.512\div W$**

Where: $V_{Total\ sample}$: added the Reagent Ⅰ volume, 0.9 mL; V_{Sample}: sample volume added to reaction, 0.04 mL; V_{Total}: total reaction volume (enzymatic reaction), 0.2 mL; W:sample weight, g; T: enzymatic reaction time, 60 min; 180.16: molar mass of glucose, μg/μmol.

Precautions

What if the obtained ΔA_{Test} is too high or too low?

If ΔA_{Test} is higher than $\Delta A_{Standard}$ of the 1 mmol/L standard, the cellulase activity in the sample is too high. Dilute the sample appropriately with Reagent Ⅰ and repeat the assay. If $\Delta A_{Test} < 0.01$, increase the sample amount (soil or plant tissue weight) or decrease the Reagent Ⅰ volume, then repeat the assay.

Typical Data

Typical standard curve-data provided for demonstration purposes only. A new standard curve must be generated for each assay.

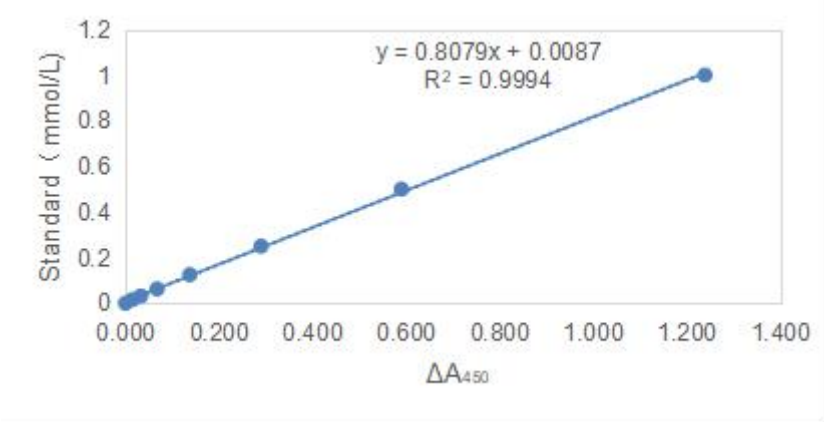

Figure 1. Standard curve of cellulase activity assay.

Examples:

1. Test 0.1 g soil, prepared the sample following the above protocol and measured with the 96-well microplate:

$\Delta A_{Test}=A_{Test}-A_{Control}=0.271-0.179=0.092$.

Substitute the ΔA_{Test} into the equation to obtain the y=0.083.

2. Calculated by fresh weight of samples:

Cellulase activity (U/g)=y×13.512÷W=11.215 U/g.

Recommended Products

Catalog No.	Product Name
KTB4038	CheKine™ Micro Soil Exo-β-1,4-Glucanase (S-C1) Activity Assay Kit
KTB1360	CheKine™ Micro Reducing Sugar (RS) Assay Kit
KTB1322	CheKine™ Micro β-glucosidase (β-GC) Activity Assay Kit
KTB4022	CheKine™ Micro 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. For your safety and health, please wear a lab coat and disposable gloves.