CheKine™ Micro 1,4-β-D-Glucan Cellobilhydrolase (C1) Activity Assay Kit

Cat #: KTB3019 Size: 48 T/48 S 96 T/96 S

	Micro 1,4-β-D-Glucan Cellobilhydrolase (C1) Activity Assay Kit		
REF	**Cat #:** KTB3019	LOT	**Lot #:** Refer to product label
	Detection range: 40-600 μg/mL		**Sensitivity:** 40 μg/mL
	Applicable sample: Animal Tissues, Fungus		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

1,4-β-D-Glucan Cellobilhydrolase (C1, EC3.2.1.91) exists in bacteria, fungi and animals, and is one of the components of cellulase system. C1 catalyzes the release of cellobiose and glucose from the non-reducing end of polysaccharide chain. CheKine™ Micro 1,4-β-D-Glucan Cellobilhydrolase (C1) Activity Assay Kit can detect biological samples such as soli. In this kit, the content of reducing sugar produced by the degradation of microcrystalline cellulose catalyzed by C1 was determined by 3,5-dinitrosalicylic acid method. The generated brownish-red amino compound has characteristic light absorption at 540 nm, and the increase rate of light absorption at 540 nm is proportional to the activity of C1 in a certain range.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	120 mL	4℃
Reagent Ⅰ	6 mL	12 mL	4℃, protected from light
Reagent Ⅱ	30 mL	60 mL	4℃, protected from light
Standard	Powder×1 vial	Powder×1 vial	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 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, freezing centrifuge
- Deionized water
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light. It is normal for Reagent Ⅰ to be turbid or appear white precipitate. Shake it several times before use and mix well.

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

Note: Reagent Ⅱ 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 10 mg/mL glucose Standard. The remaining reagent can be stored at 4℃ for 2 weeks. Using 10 mg/mL glucose standard solution, prepare standard curve dilution as described in the table:

Num.	Standard Volume (μL)	Deionized Water (μL)	Concentration (μg/mL)
Std.1	60 μL of 10 mg/mL Standard	940	600
Std.2	600 μL of Std.1 (600 μg/mL)	300	400
Std.3	600 μL of Std.2 (400 μg/mL)	600	200
Std.4	600 μL of Std.3 (200 μg/mL)	600	100
Std.5	800 μL of Std.4 (100 μg/mL)	200	80
Std.6	500 μL of Std.5 (80 μg/mL)	500	40

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

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. Plant tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize or mortar on ice. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Fungus: Collect 5×10^6 fungus into the centrifuge tube, wash fungus with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the fungus 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

Note: 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 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 (μL)	Control Tube (μL)	Background Tube (μL)	Standard Tube (μL)	Blank Tube (μL)
Sample	10	10	0	0	0
Reagent Ⅰ	100	0	100	0	0
Deionized Water	0	100	10	0	0
Mix well, and accurate response at 37℃ for 2 h.				0	0

Standard	0	0	0	110	0
Deionized Water	0	0	0	0	110
Reagent II	200	200	200	200	200

Mix well, bathe in water at 100℃ for 10 min (cover tightly to prevent water loss), cool down with running water to room temperature and centrifuge at 5,000 g for 5 min at room temperature, 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 Background Well is marked as $A_{Background}$, the Control Well is marked as $A_{Control}$, and the Test Well is marked as A_{Test}. Finally calculate $\Delta A_{Test}=(A_{Test}-A_{Control})-(A_{Background}-A_{Blank})$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: The Background Well, 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 600 μg/mL of $\Delta A_{Standard}$, the sample can be appropriately diluted with Extraction Buffer, 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 (μg/mL).

2. Calculation of the C1 activity

(1) Calculated by protein concentration

Active unit definition: At 37℃, 1 μg glucose is produced per min in 1 mg tissue protein reaction system is defined as a unit of enzyme activity.

C1 (U/mg prot)=$x\times V_{Reaction}\div V_{sample}\times V_{Total\ sample}\div Cpr\div T$**$=0.092x\div Cpr$**

(2) Calculated by fresh weight of samples

Active unit definition: At 37℃, 1 μg glucose is produced per min in 1 g tissue reaction system is defined as a unit of enzyme activity.

C1 (U/g fresh weight)=$x\times V_{Reaction}\div V_{sample}\times V_{Total\ sample}\div W\div T$**$=0.092x\div W$**

(3) Calculated by fungus

Active unit definition: At 37℃, 1 μg glucose is produced per min in 10^4 fungus reaction system is defined as a unit of enzyme activity.

C1 (U/10^4 fungus)=$x\times V_{Reaction}\div V_{sample}\times V_{Total\ sample}\div N\div T$**$=0.092x\div N$**

$V_{Reaction}$: Enzymatic reaction volume 0.11 mL; V_{sample}: added sample volume, 0.01 mL; $V_{Total\ sample}$: added Extraction Buffer volume, 1 mL; T: reaction time, 2 h=120 min; Cpr: sample protein concentration, mg/mL; W: Sample weight, g; N: Fungus counts in tens of thousands.

Typical Data

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

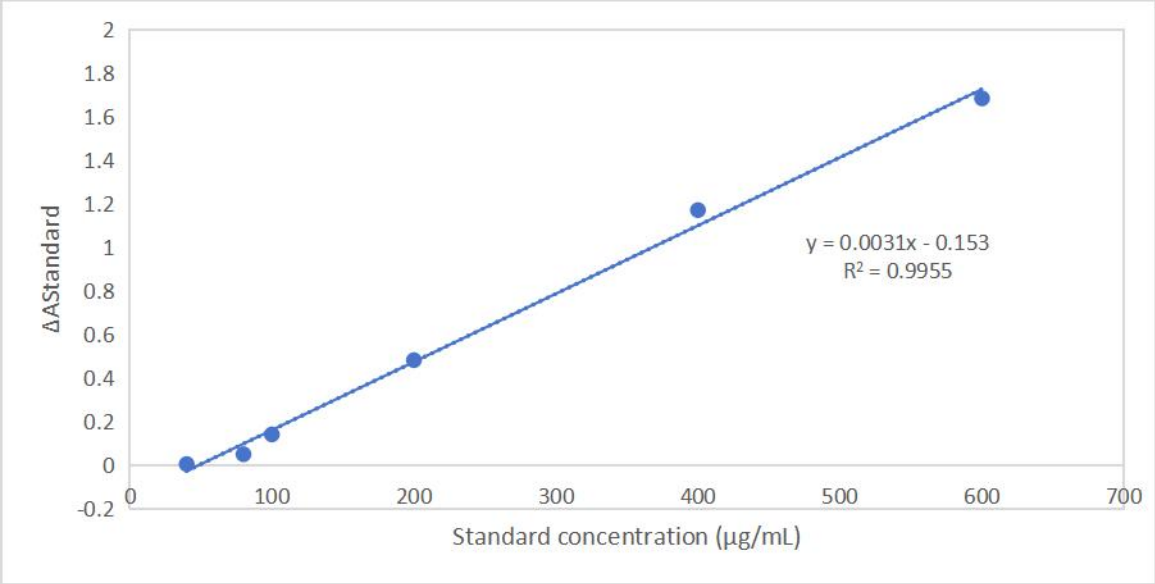

Figure 1. Standard curve of C1 activity.

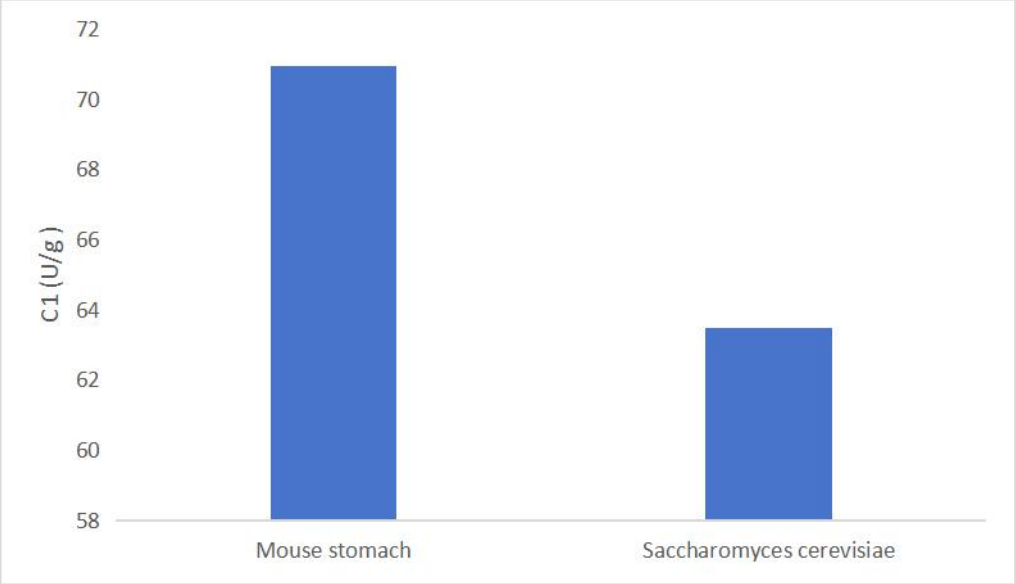

Figure 2. Determination C1 activity in mouse stomach and saccharomyces cerevisiae by this assay kit.

Recommended Products

Catalog No.	Product Name
KTB1150	CheKine™ Micro Peroxidase (POD) Activity Assay Kit
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Activity Assay Kit
KTB1040	CheKine™ Micro Catalase (CAT) Content 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.