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CheKine™ Micro β-1,3-Glucanase (β-1,3-GA) Activity Assay Kit

Cat #: KTB1325 Size: 48 T/96 T

	Micro β-1,3-Glucanase (β-1,3-GA) Activity Assay Kit		
REF	**Cat #:** KTB1325	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Bacteria or Cells, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

β-1,3-glucanase (β-1,3-GA) mainly exists in plants and catalyzes the hydrolysis of β-1, 3-glucoside bond. A large number of β-1,3-GA can be induced by plant infection or other adverse conditions. Therefore, β-1,3-GA activity assay has been widely used in plant pathology and stress physiology studies. CheKine™ Micro β-1,3-Glucanase (β-1,3-GA) Activity Assay Kit can be used to detect biological samples such as animal and plant tissues, bacteria or cells, serum or plasma. In the kit, β-1,3-GA hydrolyzes laminarin and inner cuts β-1, 3-glucoside bond to produce reducing terminus. The enzyme activity is calculated by measuring the rate of reducing sugar production.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	1	1	4℃, protected from light
Reagent Ⅱ	15 mL	30 mL	4℃, protected from light
Standard	1	1	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 550 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL eppendorf tube
- Water bath pot, cryogenic centrifuge machine
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

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

Reagent I: Prepared before use. 48 T add 1 mL deionized water, 96 T add 2 mL deionized water to fully dissolve. The prepared reagent can be stored at 4℃, protected from light for 4 weeks.

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

Stardard： Prepared before use. add 1 mL deionized water to fully dissolve to generate a 10 mg/mL glucose standard solution, store at 4℃ and use within 2 weeks.

Standard preparation: Use the 10 mg/mL glucose standard solution and further dilute it to the standard as shown in the following table:

Num.	Standard Volume (μL)	Deionized Water (μL)	Concentration (mg/mL)
Std.1	100 μL 10 mg /mL Standard	900	1
Std.2	160 μL of Std.1 (10 mg/mL)	40	0.8
Std.3	120 μL of Std.1 (10 mg/mL)	80	0.6
Std.4	80 μL of Std.1 (10 mg/mL)	120	0.4
Std.5	40 μL of Std.1 (10 mg/mL)	160	0.2

Notes: Always prepare fresh Standards per use; Diluted Std. 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. Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 12,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Bacteria or Cells: Collect 5×10^6 bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 30 times (power 20% or 200 W, ultrasonic 3 s, interval 7 s). Centrifuge at 12,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Plasma, Serum or other Liquid samples: Direct detection.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 550 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Sample measurement. (The following operations are operated in the 1.5 mL eppendorf tube)

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)	Control Well (μL)
Sample	0	0	35	35
Standard	0	35	0	0
Deionized water	70	35	0	35
Reagent Ⅰ	0	0	35	0
Mix thoroughly, put in 37℃ water bath for 60 min				
Reagent Ⅱ	230	230	230	230

3. Mix thoroughly, boiling water bath for 5 min (cover tightly to prevent water loss), add 200 μL to micro glass cuvette/96 well flat-bottom plate, detect the absorbance at 550 nm after cooling with running water. The Blank Well is recorded as A_{Blank}, the standard Well is marked as $A_{Standard}$, the Test Well is marked as A_{Test}, and the Control Well is marked as $A_{Control}$. Finally calculate Δ

$A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note:The Blank Well and the Standard Well only need to be done 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.001, increase the sample quantity appropriately. If ΔA is greater than 2, the sample 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 y=kx+b. The determination of ΔA_{Test} is brought into the equation to get x(mg/mL).

2. Calculation of the β-1,3-GA activity

(1) Calculated by sample protein concentration

Unit definition: One unit of β-1,3-GA activity is defined as the amount of enzyme that per milligram of protein oxidation 1 nmoL of NADH per minute in the reaction system.

β-1,3-GA (U/mg prot)=$(x\times V_{Sample})\div(V_{Sample}\times Cpr)\div T$**=x÷Cpr**

(2) Calculated by fresh weight of samples

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 mg of reducing sugar per hour every gram of sample.

β-1,3-GA (U/g fresh weight)=$(x\times V_{Sample})\div(V_{Sample}\div V_{Extraction}\times W)\div T$**=x÷W**

(3) Calculated by bacteria or cells

Unit definition: One unit of β-1,3-GA activity is defined as the amount of enzyme that per 10^4 cells oxidation 1 nmoL of NADH per minute in the reaction system.

β-1,3-GA (U/10^4 cell)=$(x\times V_{Sample})\div(V_{Sample}\div V_{Extraction}\times 500)\div T$**=0.002×x**

(4) Calculated by volume of liquid samples

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 mg of reducing sugar per hour every 10 thousand bacteria or cells.

β-1,3-GA (U/mL)=$[x\times V_{Sample}]\div V_{Sample}\div T$**=x**

V_{Sample}: Sample volume added to the reaction system, 0.035 mL; $V_{Extraction}$: Volume of added Extraction Buffer, 1mL; T: The reacti, 60 min=1 h; Cpr: Sample protein concentration, mg/mL; W: Sample weight, g; 500: Bacteria or cell amount, 5 million.

Typical Data

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

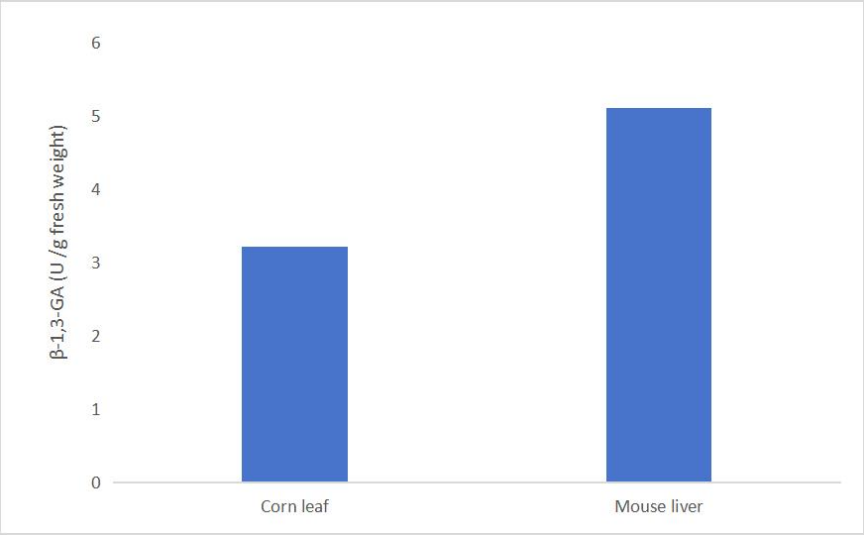

Figure 1. Determination of β-1,3-GA activity in corn leaf mouse liver by this kit.

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-glucosidase (α-GC) Activity Assay Kit
KTB1420	CheKine™ Micro β-glucosidase (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.