CheKine™ Micro β-glucosidase (β-GC) Activity Assay Kit

Cat #: KTB1322 Size: 48 T/96 T

	Micro β-Glucosidase Activity Assay Kit		
REF	**Cat #:** KTB1322	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and Plant Tissues, Bacteria, Serum (Plasma)		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

β-Glucosidase (β-GC, EC 3.2.1.21) is widely present in animals, plants, microorganisms, and cultured cells, catalyzing β-Glycoside bond hydrolysis has multiple physiological effects: in the glycosylation of cellulose, β-GC is responsible for further hydrolyzing cellulose disaccharides and cellulose oligosaccharides to produce glucose; β-GC hydrolyzes terpene aroma precursors, causing glycosidic bond states to become free states, thereby producing aroma; β-GC can hydrolyze wild cherry blossom glycoside in plants, release HCN, and prevent insect feeding. β-GC decomposition of p-nitrobenzene-β-D-glucopyranoside generates p-nitrophenol, which has a maximum absorption peak at 400 nm, and is calculated by measuring the rate of increase in absorbance value β-GC activity.

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	-20℃, protected from light
Reagent Ⅱ	8 mL	15 mL	4℃
Reagent Ⅲ	8 mL	15 mL	4℃
Standard	1 mL	1 mL	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 400 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Thermostatic water bath, ice maker, centrifuge, ultrasonic homogenizer
- Deionized water
- Mortar or homogenizer

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Reagent Preparation

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

Reagent Ⅰ: Prepared before use. Add 6 mL deionized water for 48 T and 12 mL deionized water for 96 T to fully dissolve. Unused reagents should be packaged at -20℃ and stored in dark for 4 weeks and avoid repeated freeze-thaw cycles.

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

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

Standard: 5 µmol/mL p-nitrophenol standard solution. Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Standard preparation: Use 5 µmol/mL p-nitrophenol standard solution, prepare standard curve dilution as described in the table.

Num.	Standard Volume	Deionized Water Volume (µL)	Concentration (nmol/mL)
Std.1	20 µL 5 µmol/mL Standard	980	100
Std.2	500 µL of Std.1 (100 nmol/mL)	500	50
Std.3	500 µL of Std.2 (50 nmol/mL)	500	25
Std.4	500 µL of Std.3 (25 nmol/mL)	500	12.5
Std.5	500 µL of Std.4 (12.5 nmol/mL)	500	6.25
Std.6	500 µL of Std.5 (6.25 nmol/mL)	500	3.125
Std.7	500 µL of Std.6 (3.125 nmol/mL)	500	1.563
Blank	0	500	0 (Blank Tube)

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 15,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Bacteria: Collect 5×10^6 bacteria into the centrifuge tube, wash bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 15,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Serum (Plasma): Test directly. If the solution is turbid, the supernatant should be taken for determination after centrifugation.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 400 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)	Standard Tube (µL)
Reagent Ⅰ	120	0	0
Reagent Ⅱ	150	150	0
Deionized Water	0	120	0

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Sample Supernatant	30	30	0
Fully mix well, put it in an accurate water bath at 37℃ for 30 min, then immediately put it in a water bath at 95℃ for 5 min (cover tightly to prevent water loss), fully mix well after cooling (to ensure constant concentration), centrifuge at 8,000 g at 4℃ for 5 min, and take the supernatant (add the following reagents to a 1 mL microglass cuvette or a 96-well plate)			
Standard	0	0	70
Supernatant	70	70	0
Reagent III	130	130	130

3. After fully mixing and standing at room temperature for 2 min, the absorbance was measured at 400 nm. The absorbance of test well, control well, standard well and blank well were recorded as A_{Test}, $A_{Control}$, $A_{Standard}$ and A_{Blank}. Calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Standard curve and blank tube only need to be done once or twice. Before the experiment, it is suggested that 2-3 samples with large expected differences should be selected for pre-experiment. If A_{Test} is greater than 0.4, the sample supernatant can be further diluted by Extraction Buffer, and the calculation result should be multiplied by the dilution multiple.

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, get the standard equation y=kx+b, and bring the ΔA_{Test} into the equation to get the x value (nmol/mL).

2. Calculation of β-GC activity:

(1) Calculated by protein concentration

Active unit definition: The production of 1 nmol p-nitrophenol per min in 1 mg tissue protein is defined as a unit of enzyme activity.

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

(2) Calculated by sample fresh weight

Active unit definition: The production of 1 nmol p-nitrophenol per min in 1 g tissue is defined as a unit of enzyme activity.

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

(3) Calculated by bacteria number

Active unit definition: The production of 1 nmol p-nitrophenol per min in 10^4 bacteria is defined as a unit of enzyme activity.

β-GC (U/10^4)=$(x\times V_{Total})\div(500\times V_{Sample}\div V_{Total\ Sample})\div T$=**x÷500÷3**

(4) Calculated by volume of liquid samples

Active unit definition: The production of 1 nmol p-nitrophenol per min in 1 mL liquid samples is defined as a unit of enzyme activity.

β-GC (U/mL)=$(x\times V_{Total})\div(V_{Sample}\div V_{Total\ Sample})\div T$=**x÷3**

V_{Total}: total reaction volume, 0.3 mL; V_{Sample}: sample volume added, 0.03 mL; $V_{Total\ Sample}$: Extraction Buffer volume added, 1 mL; Cpr: sample protein concentration, mg/mL; W: sample weight, g; 500: Total number of bacteria, 5×10^6. T: reaction time, 30 min.

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Typical Data

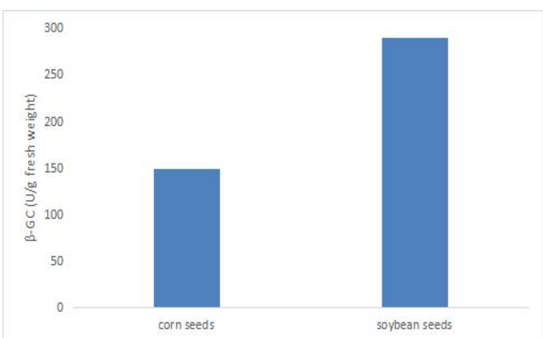

Figure 1. Determination β-GC activity in corn seeds and soybean seeds by this assay kit

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-Glucosidase Activity Assay Kit
KTB1121	CheKine™ Pyruvate Acid (PA) Colorimetric Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.

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