（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K822-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（395-410 nm）

Detection range：0.06-6.79 U/L

Elabscience®β-Glucosidase (β-GC) Activity Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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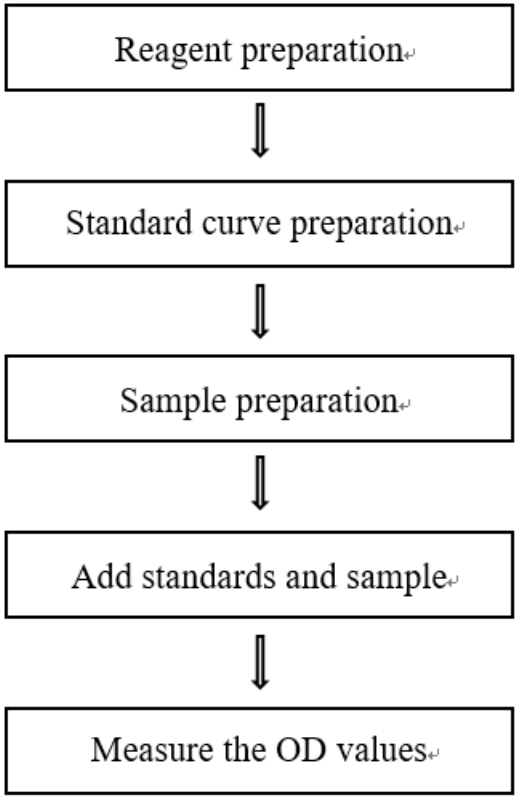
Assay summary



Intended use

This kit can be used to detect the β-glucosidase (β-GC) activity in tissues and fungus samples.

Detection principle

β-glucosidase (β-GC), also known as β-glucoside hydrolase, can efficiently hydrolyze the terminal non-reducing β-D-glucoside bonds in various glycoside compounds, thereby releasing β-D-glucose and corresponding ligands. The enzyme is widely present in nature, including the seeds of various plants, the small intestine and other organs of animals, and microorganisms.

β-GC is a very important industrial enzyme with various applications. In the field of medicine, β-GC catalyzes the degradation of glycoside groups to inhibit the expansion of cancer cells. In terms of diet, β-GC catalyzes the production of anti-cancer molecules isoflavones in soybean. In the energy field, β-GC is one of the key enzymes in the conversion of cellulose to glucose.

β-GC catalyzed the reaction of substrate to produce chromogenic substances at the wavelength of 400 nm, the maximum absorption of β-GC enzyme activity was reflected by measuring the OD value at 400 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution	50 mL ×1 vial	50 mL ×2 vials	-20 ℃, 12 months
Reagent 2	Buffer	25 mL ×1 vial	50 mL ×1 vial	-20 ℃, 12 months
Reagent 3	Substrate	2.5 mL ×1 vial	5 mL ×1 vial	-20 ℃, 12 months, shading light
Reagent 4	10 mmol/L Standard Solution	1 mL ×1 vial	2 mL ×1 vial	-20 ℃, 12 months, shading light
	Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (395-410 nm, optimum wavelength: 400 nm), Incubator (37℃)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of working solution:

For each well, prepare 180 μL of working solution (mix well 36 μL of substrate and 144 μL of buffer). The working solution should be protected from light and used up within 1 day.

③ The preparation of 200 μmol/L standard solution:

Before testing, please prepare sufficient 200 μmol/L standard solution according to the test wells. For example, prepare 2500 μL of 200 μmol/L standard solution (mix well 50 μL of 10 mmol/L standard solution and 2450 μL of buffer). The 200 μmol/L standard solution should be prepared on spot and protected from light.

④ The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 200 μmol/L standard solution with buffer diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 40, 60, 80, 120, 140, 160, 200 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**40**	**60**	**80**	**120**	**140**	**160**	**200**
200 μmol/L Standard (μL)	0	100	150	200	300	350	400	500
Buffer (μL)	500	400	350	300	200	150	100	0

Sample preparation

① Sample preparation:

Tissues and fungus samples:

① Harvest the amount of tissue or fungus needed for each assay (initial recommendation 20 mg).

② Wash tissue or fungus in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue or fungus in 180 μL extraction solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M; E-BC-K168-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Apple seeds homogenization	50-60
10% Spinage tissue homogenization	1
10% Shii-take tissue homogenization	3-5
10% Grapefruit seeds homogenization	1
10% Corn tissue homogenization	1
10% Mushroom tissue homogenization	1
10% Cabbage tissue homogenization	1
10% Mouse small intestine tissue homogenization	1
10% Pear seeds homogenization	2-4

Note: The diluent is extraction solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard wells: Add 200 μL of standard to the corresponding wells.
Sample wells: Add 20 μL of sample to the corresponding wells.

② Add 180 μL of working solution to sample wells.

③ Mix fully with microplate reader for 5 s and measure the OD value (A_1) of sample wells at 400 nm.

④ Incubate at 37 ℃ for 30 min and measure the OD value (A_2) of each well at 400 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

Tissues and fungus samples:

Definition: The amount of enzyme in 1 g sample protein that hydrolyze the substrate to produce 1 μmol product in 1 minute at 37°C is defined as 1 unit.

$$\beta\text{-GC activity (U/gport)} = (\Delta A_{400} - b) \div a \div T \times f \div C_{pr} \times 10$$

[Note]

ΔA_{400}: $\Delta A_{400} = A_2 - A_1$.

f: Dilution factor of sample before test.

t: Reaction time, 30 min.

C_{pr}: Concentration of protein in sample, gprot/L.

10: When add the sample to the reaction system, the protein concentration is diluted 10 times.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three spinage samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	1.50	2.50	3.20
%CV	2.8	3.2	4.5

Inter-assay Precision

Three spinage samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	1.50	2.50	3.20
%CV	2.0	9.7	5.6

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 102%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	50	100	150
Observed Conc. (μmol/L)	49.5	102	156
Recovery rate (%)	99	102	104

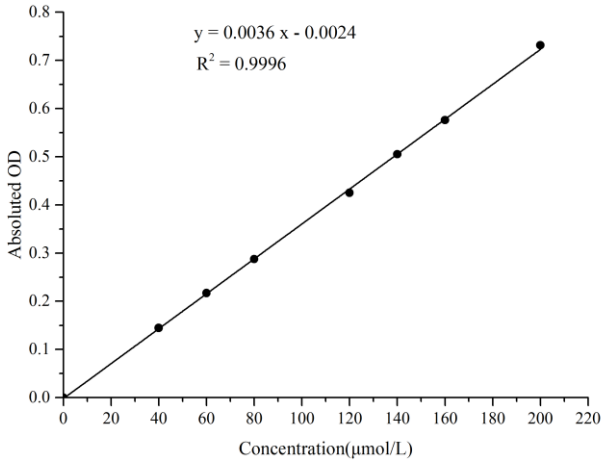
Sensitivity

The analytical sensitivity of the assay is 0.06 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only.

Concentration (μmol/L)	**0**	**40**	**60**	**80**	**120**	**140**	**160**	**200**
OD value	0.050	0.193	0.267	0.338	0.476	0.557	0.627	0.782
	0.048	0.194	0.265	0.335	0.472	0.552	0.623	0.779
Average OD	0.049	0.194	0.266	0.337	0.474	0.555	0.625	0.781
Absoluted OD	0	0.145	0.217	0.288	0.425	0.506	0.576	0.732



Appendix II Example Analysis

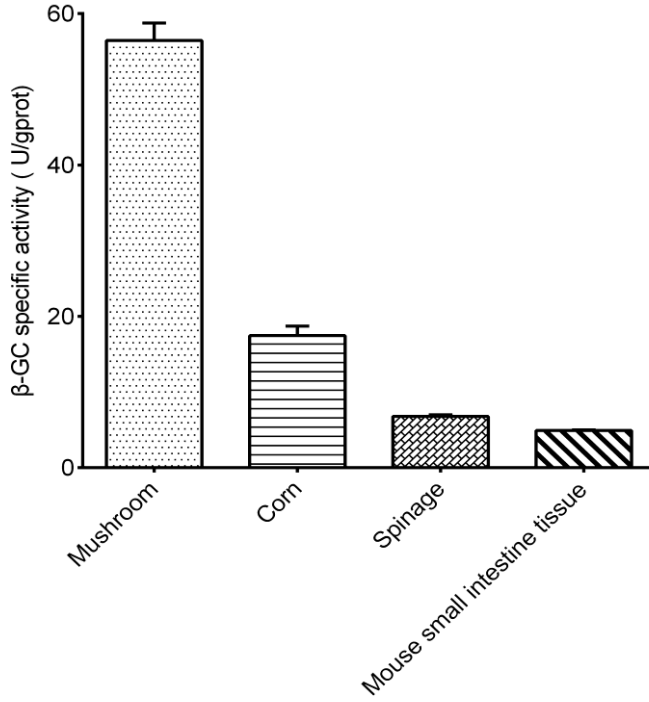
Example analysis：

Take 20 μL of 10% mouse small intestine tissue homogenization and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0036 x - 0.0019, the average A_1 of the sample is 0.240, the average A_2 of the sample is 0.644, $\Delta A_{400} = A_2 - A_1 = 0.644 - 0.240 = 0.404$, the concentration of protein in sample is 7.75 gprot/L and the calculation result is:

$$\beta\text{-GC activity (U/gprot)} = (0.404 + 0.0019) \div 0.0036 \div 30 \times 1 \div 7.75 \times 10 = 4.85 \text{ U/gprot}$$

Detect 10% mushroom tissue homogenization (the concentration of protein in sample is 0.69 gprot/L), 10% corn tissue homogenization (the concentration of protein in sample is 1.24 gprot/L), 10% spinage tissue homogenization (the concentration of protein in sample is 1.10 gprot/L) and 10% mouse small intestine tissue homogenization (the concentration of protein in sample is7.75 gprot/L) according to the protocol, the result is as follows：


β-GC specific activity (U/gprot)
60
40
20
0
Mushroom
Corn
Spinage
Mouse small intestine tissue

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.