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

Catalog No：E-BC-K821-M

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

Measuring instrument：Microplate reader（390-405 nm）

Detection range：0.35-33.43 U/L

Elabscience®α-Glucosidase (α-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
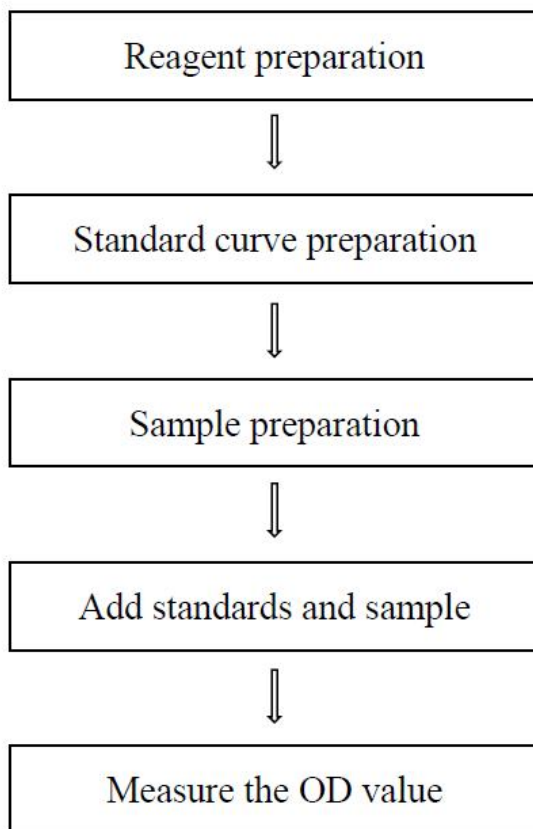Email: techsupport@elabscience.com
Website: www.elabscience.com

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

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Intended use

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

Detection principle

α-Glucosidase (α-GC), also known as α-D-glucoside hydrolase, is widely distributed in nature, with a wide variety of species and different properties, and exists in almost all organisms. It has important physiological functions in carbohydrate metabolism in animals, plants and microorganisms. If α-GC is deficient, it leads to severe glycogen metabolism disorders and excessive glycogen accumulation, which can cause Pompah's disease (an autosomal recessive inherited glycogen storage disease, also known as glycogenosis type II). Long-term low α-GC activity in human tissues can cause muscle fiber destruction and muscle atrophy.

The detection principle of this kit is that the chromogenic substance generated by the substrate reaction catalyzed by α-glucosidase has the maximum absorption at 400 nm, and the enzyme activity of α-GC can be reflected by measuring its 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°C, 12 months
Reagent 2	Buffer Solution	7 mL × 1 vial	14 mL × 1 vial	-20°C, 12 months
Reagent 3	Substrate	0.5 mL × 1 vial	1 mL × 1 vial	-20°C, 12 months, shading light
Reagent 4	10 mmol/L Standard Solution	1 mL × 1 vial	1 mL × 2 vials	-20°C, 12 months, shading light
	Microplate	48 wells	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 (390-405 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 120 μL of working solution (mix well 8 μL of substrate and 112 μL of buffer solution). The working solution should be used up within 1 day.

③ The preparation of 1 mmol/L standard solution：

Before testing, please prepare sufficient 1 mmol/L standard solution according to the test wells. For example, prepare 1000 μL of 1 mmol/L standard solution (mix well 100 μL of 10 mmol/L standard solution and 900 μL of double distilled water). The 1 mmol/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 1 mmol/L standard solution with double distilled water diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.7, 1 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.7**	**1**
1 mmol/L Standard (µL)	0	20	40	60	80	100	140	200
Double distilled water (µL)	200	180	160	140	120	100	60	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 12000×g for 15 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% Mouse small intestine tissue homogenization	1-2
10% Rat kidney tissue homogenization	1-2
10% Oranges seeds homogenization	1
10% Mouse kidney tissue homogenization	1-2
10% Apricot abalone mushroom tissue homogenization	1
10% Pear seeds homogenization	1
10% Corn tissue homogenization	1

| 10% Apple seeds homogenization | 1 |

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 20 μL of standard to the corresponding wells.
   Sample wells: Add 20 μL of sample to the corresponding wells.
② Add 120 μL of buffer solution to standard wells. Add 120 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°C 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 (A_2) 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.

$$\frac{\alpha\text{-GC activity}}{\text{(U/gport)}} = (\Delta A_{400} - b) \div a \div T \times f \div C_{pr} \times 1000^*$$

[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.

1000*: 1 mmol/L= 1000 μmol/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three rat kidney 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)	5.00	15.00	20.00
%CV	4.4	5.0	5.7

Inter-assay Precision

Three rat kidney 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)	5.00	15.00	20.00
%CV	4.2	4.6	6.6

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 105%.

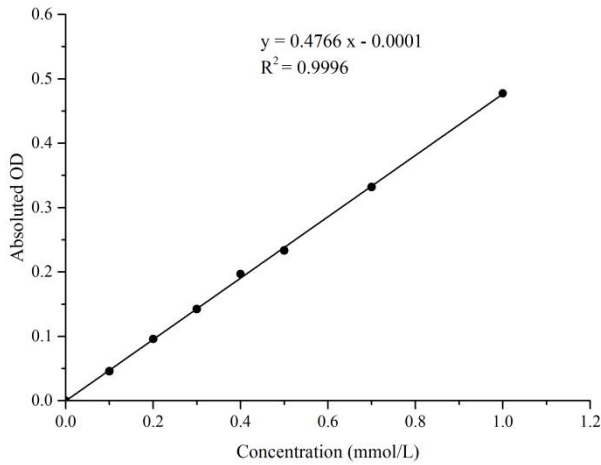|	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.15	0.35	0.60
Observed Conc. (mmol/L)	0.15	0.37	0.66
Recovery rate (%)	98	106	110

Sensitivity

The analytical sensitivity of the assay is 0.35 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 (mmol/L)	0	0.1	0.2	0.3	0.4	0.5	0.7	1.0
OD value	0.040	0.086	0.136	0.184	0.256	0.276	0.380	0.521
	0.041	0.087	0.137	0.182	0.219	0.272	0.365	0.515
Average OD	0.041	0.087	0.137	0.183	0.238	0.274	0.373	0.518
Absoluted OD	0	0.046	0.096	0.143	0.197	0.234	0.332	0.478

$y = 0.4766 x - 0.0001$
$R^2 = 0.9996$

Appendix II Example Analysis

Example analysis：

Take 10% mouse small intestine tissue homogenization, dilute for 2 times, take 20 μL of diluted sample and carry the assay according to the operation steps. The results are as follows:

Standard curve: $y = 0.4766 x - 0.0001$, the average A_1 of the sample is 0.095, the average A_2 of the sample is 0.407, $\Delta A_{400} = A_2 - A_1 = 0.407 - 0.095 = 0.312$, the concentration of protein in sample is 9.06 gprot/L and the calculation result is:

α-GC activity (U/gprot) = $(0.312 + 0.0001) \div 0.4766 \div 30 \div 9.06 \times 2 \times 1000 = 4.82$ U/gprot

Detect 10% rat kidney tissue homogenization (the concentration of protein in sample is 12.22 gprot/L, dilute for 2 times), 10% corn tissue homogenization (the concentration of protein in sample is 1.56 gprot/L), 10% apricot abalone mushroom tissue homogenization (the concentration of protein in sample is 0.34 gprot/L) and 10% mouse small intestine tissue homogenization (the concentration of protein in sample is 9.06 gprot/L, dilute for 2 times) according to the protocol, the result is as follows：
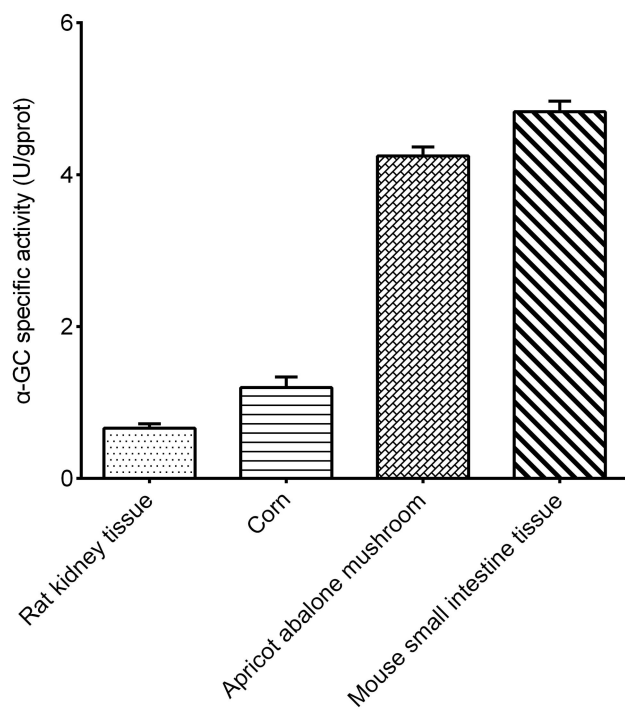
Statement

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.