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

Catalog No：E-BC-D017

Specification：48T/96T

Measuring instrument：Microplate Reader (390-410 nm)

Elabscience® Alpha-Glucosidase Inhibitor Screening 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
Tel: 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 measure the inhibitory effect of alpha-glucosidase inhibitors.

Detection principle

Alpha-glucosidase catalyzes the release of α-glucose from the non-reducing end of the substrate and promotes the absorption of glucose by the small intestine. Inhibition of α-glucosidase is an effective method for the treatment of non-insulin-dependent diabetes mellitus (NIDDM).

The detection principle of this kit: α-Glucosidase catalyzes the substrate reaction to generate colored products, whose absorbance increases at 400 nm. The addition of an inhibitor suppresses the enzymatic activity of α-glucosidase, resulting in a reduced rate of absorbance increase. The inhibition rate can be calculated based on the absorbance difference.

Kit components & storage

Item	Component	Size (48 T)	Size (96 T)	Storage
Reagent 1	Buffer Solution	15 mL × 1 vial	30 mL × 1 vial	-20°C, 12 months shading light
Reagent 2	Enzyme Reagent	0.05 mL × 1 vial	0.1 mL × 1 vial	-20°C, 12 months shading light
Reagent 3	5 mmol/L Acarbose	0.05 mL × 1 vial	0.05 mL × 1 vial	-20°C, 12 months shading light
Reagent 4	Substrate	0.11 mL × 1 vial	0.22 mL × 1 vial	-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 (390-410 nm, optimum wavelength: 400 nm), Incubator (37℃)

Reagents:

DMSO

Reagent preparation

① Equilibrate all reagents to 25°C before use.

② The preparation of enzyme working solution：

Before testing, please prepare sufficient enzyme working solution according to the test wells. For example, prepare 125 μL of enzyme working solution (mix well 5 μL of enzyme reagent and 120 μL of double distilled water). Keep enzyme working solution on ice during use. Store at -20°C for 2 days.

③ The preparation and application of acarbose working solution:

The concentration of the positive control inhibitor acarbose provided in this kit is 5 mmol/L, which can be diluted to the desired concentration with buffer solution. The IC_{50} in this kit is about 300 nmol/L, and the measured data will be different.

④ The preparation of substrate working solution:

Before testing, please prepare sufficient substrate working solution according to the test wells. For example, prepare 50 μL of substrate working solution (mix well 5 μL of substrate and 45 μL of buffer solution). Keep substrate working solution on ice protected from light during use. Store at -20°C for 2 days.

Sample preparation

It is recommended to dilute the sample with buffer solution. For sample with poor water-soluble, prepare a high-concentration stock solution in DMSO and then dilute with buffer solution. The concentration of DMSO in the solution of the compound should be less than 5%.

The key points of the assay

The volume of enzyme reagent and 5 mmol/L acarbose is small, and it needs to be centrifuged before use to avoid the loss of opening the cover.

Operating steps

① Blank well: Add 120 μL of buffer solution to the corresponding wells.
Total enzyme well: Add 100 μL of buffer solution to the corresponding wells.
Positive control well: Add 80 μL of buffer solution to the corresponding wells.
Sample well: Add 80 μL of buffer solution to the corresponding wells.

② Add 20 μL of enzyme working solution into total enzyme wells, positive control wells and sample wells.

③ Add 20 μL of acarbose working solution into positive control wells. Add 20 μL of sample into sample wells.

④ Add 20 μL of substrate working solution into each well.

⑤ Mix fully with microplate reader for 5 s and incubate at 37ºC for 10 min. Measure the OD value of each well at 400 nm with microplate reader.

(The positive control well determines the inhibition rate of the α-glucosidase-specific inhibitor and as a reference only. Positive control wells can be selectively detected.)

Calculation

$$\text{Inhibition Rate (\%)} = (\Delta A_1 - \Delta A_2) \div \Delta A_1 \times 100\%$$

[Note]

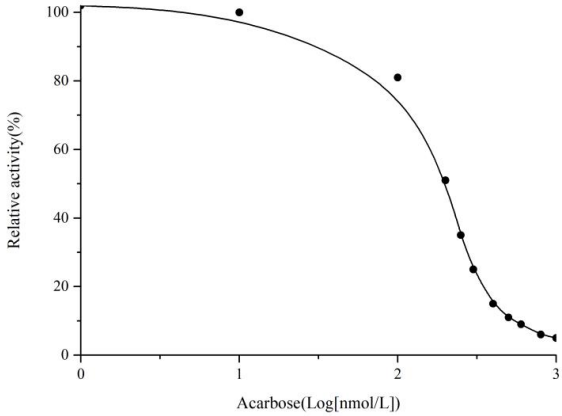
ΔA_1: $\Delta A_1 = OD_{total} - OD_{blank}$.

ΔA_2: $\Delta A_2 = OD_{sample} - OD_{blank}$.

Appendix I Performance Characteristics

Inhibition curve

The effect of α-glucosidase inhibitor screening kit for the detection of inhibitor acarbose.



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.