



## **TECHNICAL MANUAL**

### **Alpha-Glucosidase Inhibitor Screening Kit**

- **SKU CODE:** MAES0495
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

## 1. Intended use

This kit can be used to measure the inhibitory effect of alpha-glucosidase inhibitors.

## 2. Detection principle

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

The detection principle of this kit:  $\alpha$ -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  $\alpha$ -glucosidase, resulting in a reduced rate of absorbance increase. The inhibition rate can be calculated based on the absorbance difference.

## 3. Kit components & storage

Item	Component	Size (48 T)	Size (96 T)	Storage
Reagent 1	Buffer Solution	15 mL $\times$ 1 vial	30 mL $\times$ 1 vial	-20 °C, 12 months, shading light
Reagent 2	Enzyme Reagent	0.05 mL $\times$ 1 vial	0.1 mL $\times$ 1 vial	-20 °C, 12 months, shading light
Reagent 3	5 mmol/L Acarbose	0.05 mL $\times$ 1 vial	0.05 mL $\times$ 1 vial	-20 °C, 12 months shading light
Reagent 4	Substrate	0.11 mL $\times$ 1 vial	0.22 mL $\times$ 1 vial	-20 °C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

## 4. Materials prepared by users

### Instruments:

Microplate reader (390-410 nm, optimum wavelength: 400 nm), Incubator (37 °C)

**Reagents:**

DMSO

## 5. Reagent preparation

1. Equilibrate all reagents to 25 °C before use.
2. **Preparation of enzyme working solution:** Before testing, 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. **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<sub>50</sub> in this kit is about 300 nmol/L, and the measured data will be different.
4. **Preparation of substrate working solution:** Before testing, 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.

## 6. Sample preparation

It is recommended to dilute the sample with buffer solution. For samples with poor water-solubility, 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%.

## 7. 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 loss when opening the cover.

## 8. 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  $\mu\text{L}$  of enzyme working solution into total enzyme wells, positive control wells and sample wells.
3. Add 20  $\mu\text{L}$  of acarbose working solution into positive control wells. Add 20  $\mu\text{L}$  of sample into sample wells.
4. Add 20  $\mu\text{L}$  of substrate working solution into each well.
5. Mix fully with microplate reader for 5 s and incubate at 37  $^{\circ}\text{C}$  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  $\alpha$ -glucosidase-specific inhibitor and as a reference only. Positive control wells can be selectively detected.)

## 9. Calculation

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

[Note]

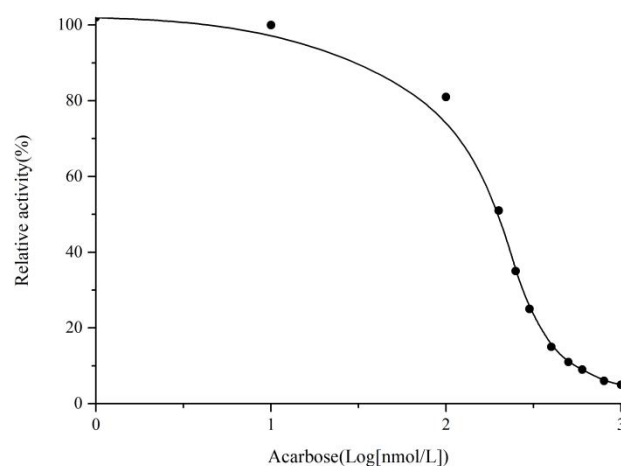
$$\Delta A_1: \Delta A_1 = \text{OD}_{\text{total}} - \text{OD}_{\text{blank}}$$

$$\Delta A_2: \Delta A_2 = \text{OD}_{\text{sample}} - \text{OD}_{\text{blank}}$$

## 10. Appendix I Performance Characteristics

Inhibition curve

The effect of  $\alpha$ -glucosidase inhibitor screening kit for the detection of inhibitor acarbose.



## 11. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

**Assay Genie 100% money-back guarantee!**

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.



**Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.**