



## **TECHNICAL MANUAL**

### **Alpha-Amylase Inhibitor Screening Kit**

- **SKU CODE:** MAES0496
- **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  $\alpha$ -amylase inhibitors.

## 2. Detection principle

$\alpha$ -Amylase in the human body can act not only on the terminal ends of starch but also on the  $\alpha$ -1,4 glucosidic bonds within starch molecules. The degradation products are glucose, maltose, and dextrin containing  $\alpha$ -1,6 glucosidic bond branch chains, which play an important role in the digestion of polysaccharide compounds in food. Inhibition of  $\alpha$ -amylase activity can reduce the digestion and absorption of starch, allowing undigested starch to enter the large intestine, promoting gastrointestinal motility, and reducing the occurrence of type 2 diabetes. This makes  $\alpha$ -amylase an important drug target for diabetes treatment.

Detection principle:  $\alpha$ -Amylase catalyzes the substrate reaction to generate colored products, whose absorbance increases at 405 nm. The addition of an inhibitor suppresses  $\alpha$ -amylase activity, 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	Powder $\times$ 2 vials	Powder $\times$ 4 vials	-20°C, 12 months, shading light
Reagent 3	5 mmol/L Acarbose	0.1 mL $\times$ 1 vial	0.1 mL $\times$ 1 vial	-20°C, 12 months, shading light
Reagent 4	Substrate	0.2 mL $\times$ 1 vial	0.4 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 (400-410 nm, optimum wavelength: 405 nm), Incubator (37°C)

**Reagents:**

DMSO

## 5. Reagent preparation

1. Equilibrate all reagents to 25°C before use.
2. **Preparation of enzyme working solution:** Dissolve one vial of enzyme reagent with 1.5 mL of buffer solution and mix well to dissolve. Keep enzyme working solution on ice during use, protected from light. Store at -20°C for up to 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 approximately 10 µmol/L, and measured data may vary.
4. **Preparation of substrate working solution:** Before testing, prepare sufficient substrate working solution according to the number of test wells. For example, prepare 75 µL of substrate working solution by mixing 5 µL of substrate with 70 µL of buffer solution. Keep substrate working solution on ice protected from light during use. Store at -20°C for up to 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 final compound solution should be less than 5%.

## 7. The key points of the assay

The volume of 5 mmol/L acarbose is small and needs to be centrifuged before use to avoid loss when opening the vial.

## 8. Operating steps

1. **Add reagents to wells:** Blank well: Add 80 µL of buffer solution to the corresponding wells. Total enzyme well: Add 50 µL of enzyme working solution and 30 µL of buffer solution to the corresponding wells. Positive control well: Add 50

μL of enzyme working solution and 30 μL of acarbose working solution to the corresponding wells. Sample well: Add 50 μL of enzyme working solution and 30 μL of samples to the corresponding wells.

2. Add 50 μL of substrate working solution into each well.
3. Mix fully with microplate reader for 5 seconds and incubate at 37°C for 20 minutes. Measure the OD value of each well at 405 nm with a microplate reader. (The positive control well determines the inhibition rate of the α-amylase specific inhibitor and serves as a reference only. Positive control wells can be selectively detected. The IC50 in this kit is approximately 10 μmol/L, and measured data may vary.)

## 9. Calculation

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

[Note]

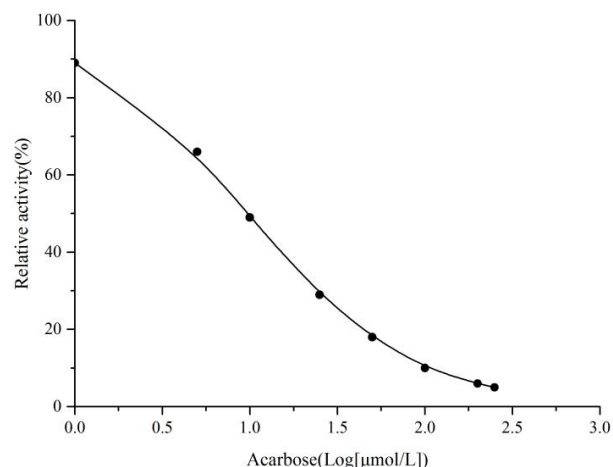
$$\Delta A_1: \Delta A_1 = OD_{\text{total}} - OD_{\text{blank}}$$

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

## 10. Appendix I Performance Characteristics

Inhibition curve

The effect of α-amylase 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.**