

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

Catalog No: E-BC-D018

Specification: 48T/96T

Measuring instrument: Microplate Reader (400-410 nm)

Elabscience® Alpha-Amylase 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:

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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 α -Amylase inhibitors.

Detection principle

α -Amylase in human body can not only act on the end of starch, but also act on the α -1,4 glucoside bond inside starch molecules. The degradation products are glucose, maltose and dextrin containing α -1,6 glucoside bond branch chain, which plays an important role in the digestion of polysaccharide compounds in food. Inhibition of α -amylase activity can not only reduce the digestion and absorption of starch, but also allow undigested and absorbed starch to enter the large intestine, promote gastrointestinal motility, and further reduce the occurrence of type 2 diabetes, which is an important drug target for the treatment of diabetes.

The detection principle of this kit: α -Amylase catalyzes the substrate reaction to generate colored products, whose absorbance increases at 405 nm. The addition of an inhibitor suppresses the activity of α -Amylase, 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 | Powder × 2 vials | Powder × 4 vials | -20°C, 12 months shading light |
| Reagent 3 | 5 mmol/L Acarbose | 0.1 mL × 1 vial | 0.1 mL × 1 vial | -20°C, 12 months shading light |
| Reagent 4 | Substrate | 0.2 mL × 1 vial | 0.4 mL × 1 vial | -20°C, 12 months shading light |
| | Microplate | 48 wells | 96 wells | No requirement |
| | Plate Sealer | 2 pieces | | |
| | Sample Layout Sheet | 1 piece | | |

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 (400-410 nm, optimum wavelength: 405 nm), Incubator (37°C)

Reagents:

DMSO

Reagent preparation

- ① Equilibrate all reagents to 25°C before use.
- ② The preparation of enzyme working solution:
Dissolve one vial of enzyme reagent with 1.5 mL of buffer solution, mix well to dissolve. Keep enzyme working solution on ice during use protected from light. 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 IC₅₀ in this kit is about 10 μmol/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 75 μL of substrate working solution (mix well 5 μL of substrate and 70 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 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 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 s and incubate at 37°C for 20 min.
Measure the OD value of each well at 405 nm with microplate reader.
(The positive control well determines the inhibition rate of the α -amylase specific inhibitor and as a reference only. Positive control wells can be selectively detected. The IC_{50} in this kit is about 10 $\mu\text{mol/L}$, and the measured data will be different.)

Calculation

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

[Note]

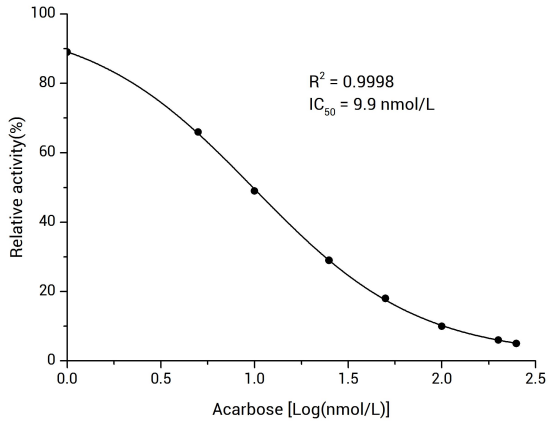
ΔA_1 : $\Delta A_1 = \text{OD}_{\text{total}} - \text{OD}_{\text{blank}}$.

ΔA_2 : $\Delta A_2 = \text{OD}_{\text{sample}} - \text{OD}_{\text{blank}}$.

Appendix I Performance Characteristics

Inhibition curve

The effect of α -Amylase 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.