TECHNICAL MANUAL

alpha-Amylase Inhibitor Screening Kit (BA0257)

- **SKU CODE:** BA0257
- **SIZE:** 400 tests (384 well format)
- **DETECTION PRINCIPLE:** Fluorometric
- **RUO:** Research-Use-Only

Revised: 07/2025

alpha-Amylase Inhibitor Screening Kit

Please read entire manual carefully before starting experiment.

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1. Key Features

Specification:

Non-radioactive assay

Applications:

For evaluation of drugs and screening potential inhibitors of α-amylase.

Procedure Time:

40 minutes

Other:

Robust and High-throughput. A Z'-factor of >0.90 was observed in a 384-well format. Can be readily automated to assay thousands of samples per day

2. Storage & Expiry

This product should be stored at -20°C up to 6 months. For detailed storage instructions of individual kit components, please refer to Section 4. The expiration date is indicated on the outer label of the kit box.

3. Product Description

Amylase belongs to the family of glycoside hydrolase enzymes that break down starch into glucose molecules by acting on α-1,4-glycosidic bonds. The α-amylases (EC 3.2.1.1) cleave at random locations on the starch chain, ultimately yielding maltotriose and maltose, glucose and "limit dextrin" from amylose and amylopectin. In mammals, α-amylase is a major digestive enzyme. Increased enzyme levels in humans are associated with salivary trauma, mumps due to inflammation of the salivary glands, pancreatitis and renal failure.

Simple, direct and automation-ready procedures for measuring αamylase inhibition are highly desirable in Research and Drug Discovery. Assay Genie's α-Amylase Inhibitor Screening Kit utilizes fluorescence polarization (FP), a highly reliable and robust technique that significantly reduces background matrix interferences, enabling the effective identification of potential α-amylase inhibitors. In this assay, αamylase cleaves a fluorescent amylose substrate. The decrease in FP is directly proportional to the α-amylase activity in the sample. Inhibition is therefore determined by the increase in FP (λex/em = 485/520 nm).

4. Kit Contents

No	Component Name	Size (400T)	Storage
1	Assay Buffer	10 mL	Stored at -20°C
2	Substrate	12 mL	Stored at -20°C
3	Amylase	30 µL	Stored at -20°C
4	Inhibitor Control	80 µL 3mM Acarbose	Stored at -20°C

Note: *All reagents must be stored according to the specified conditions listed in the table above. Do not mix reagents from different kits, as this may compromise assay performance. For reagents provided in small volumes, centrifuge briefly before use to ensure complete recovery of the contents.*

Additional materials required:

1. Microplate Reader, capable of reading FP at (λex/em = 485/520 nm).
2. Precision pipettes and disposable pipette tips. Distilled water.
3. Disposable tubes for sample dilution.
4. Absorbent paper

5. Important Notes

1. This assay kit is intended for Research Use Only. Assay Genie assumes no responsibility for any issues or legal liabilities arising from the use of this kit for clinical diagnostics or any other unauthorized purposes.
2. Please read the instructions carefully before beginning the assay. Ensure that all instruments are correctly calibrated. Strict adherence to the protocol is essential for accurate results.
3. Appropriate laboratory safety precautions must be followed, including the use of lab coats and latex gloves.
4. If the concentration of the target substance falls outside the detection range, please adjust the sample by performing further dilution or concentration as needed.
5. If your sample type is not listed in the instruction manual, we strongly recommend performing a preliminary test to confirm compatibility.
6. Experimental outcomes depend on multiple factors including reagent integrity, handling technique, and laboratory conditions. While Assay Genie guarantees the quality of our kits, we are not responsible for any loss of samples during use. We advise calculating sample requirements in advance and ensuring adequate sample volume is reserved before starting the assay.

6. Reagent Preparation

Note: *Perform assays in black flat-bottom plates. Use a plate reader capable of measuring fluorescence polarization (FP) at 485/520 nm.*

1. **Set Plate Format and Volumes**: For a 384-well plate, prepare: 30 µL enzyme, 10 µL inhibitor, 60 µL substrate and scale the volumes appropriately for other plate formats (e.g., 96-well plate).
2. **Equilibrate Reagents**: Bring all assay components to room temperature before starting. Keep enzyme on ice until use. Briefly centrifuge Amylase and inhibitor control.
3. **Prepare for Kinetic Reaction**: Since the assay is kinetic, ensure quick reagent addition. Mix reagents briefly but thoroughly to avoid variability. Use a multi-channel pipettor for consistent handling.
4. **Prepare Test Compounds**: Dissolve test compounds in H_2O or a chosen solvent (e.g., DMSO). Check the enzyme's tolerance to the selected solvent before use

7. Assay Procedure

1. **Pre-incubation**: Dilute the provided enzyme (a purified human salivary α-Amylase) 10,000-fold in Assay Buffer (or 20 mM potassium phosphate, 50 mM NaCl, pH 7.0) and use within 30 min. Transfer 15 µL of diluted enzyme into "Test" wells of a 384-well plate. For each assay run, include two additional wells, a "Control" well with 15 µL of Enzyme and a "Blank" well with 15 µL of Assay Buffer.

   To the Control and Blank wells, add 5 µL of H2O or the solvent that the test compounds are dissolved in. For example, if the test compounds are dissolved in 0.1% DMSO, add 5 µL of this solution to these wells. To the "Test" wells, add 5 µL of the test compounds. Tap plate to mix and incubate for 10 min at room temperature to allow the inhibitor to block Enzyme activity.

2. **Enzyme Activity Assay:** Add 30 µL of the Substrate to all wells. Briefly tap plate to mix. Incubate for 30 min at room temperature, protected from light. Read the FP at λex/em = 485/520 nm.

8. Data Analysis

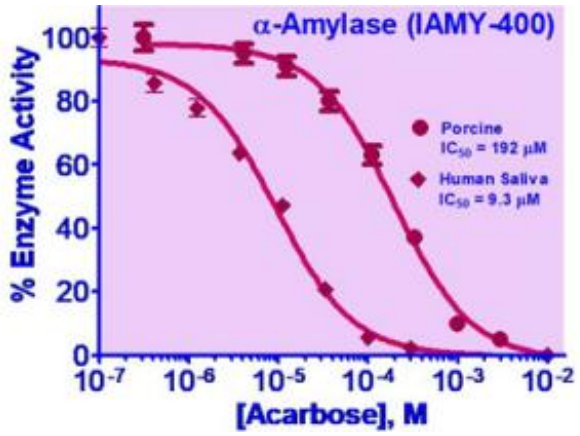
α-Amylase inhibition by a test compound is calculated as follows:

$$Enzyme\ Activity\ (\%) = \frac{FP_{Blank} - FP_{Compound}}{FP_{Blank} - FP_{Control}}\ x\ 100$$

Where $FP_{Compound}$, $FP_{Control}$, and FP_{Blank} are the fluorescence polarization values of the test compound, Control and Blank, respective

9. Typical Data

Inhibition of pure porcine and human salivary α-amylase by acarbose. IC50 values of 192 µM and 9.3 µM were observed in porcine and human enzymes, respectively. The assays were conducted in duplicate in a 384- well plate, with inhibitor control serially diluted in 3-fold increments from an initial concentration of 3 mM.



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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.