QuantiFluo™ α-Amylase Inhibitor Screening Kit (IAMY-400)

Fluorescence Polarization Inhibitor Screening Assay for α-Amylase

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 α-amylase inhibition are highly desirable in Research and Drug Discovery. BioAssay Systems' QuantiFluo™ α-Amylase Inhibitor Screening Kit utilizes fluorescence polarization (FP) to screen for potential α-amylase inhibitors. In this assay, α-amylase 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 ($\lambda_{ex/em}$ = 485/520 nm).

KEY FEATURES

Safe. Non-radioactive assay.

Fast and convenient. Homogeneous "mix-incubate-measure" type assay. Can be completed in under an hour at room temperature.

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.

APPLICATIONS

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

KIT CONTENTS (400 TESTS IN 384-WELL PLATES)

Substrate 16 mL

Bulk reagent available upon request

Storage conditions. The kit is shipped at room temperature. Store all components at -20°C upon receipt. Shelf life: 6 months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to the Material Safety Data Sheet for detailed information.

PROCEDURES

Prior to the assay, equilibrate all components to room temperature. This assay is based on a kinetic reaction. To ensure identical incubation time, addition of the Working Reagent to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. The assay can be run at room temperature. *Note: α-amylase enzyme is not included in the kit. α-Amylase is required for this inhibitor assay.*

Use a black 384-well plate and a reader capable of measuring FP at 485/520nm.

Enzyme Preparation: Enzyme can be prepared in buffer (20 mM KPi, pH 7.0, 50 mM NaCl). The following protocol is optimized for porcine α-amylase (Calzyme, Cat# 146A0100). If using a different enzyme, we recommend that you experimentally determine the optimal amount of enzyme to use per well.

Test Compound Preparation: Dissolve test compounds in a solvent of choice (e.g. DMSO). It is prudent to first test the tolerance of the solvent by the enzyme of choice. In the example below, porcine α-amylase was found to tolerate up to 5 vol% DMSO.

Inhibitor Screening in 384-Well Plate

1. Transfer 5 µL of Enzyme into separate wells of a black, flat-bottom 384-well plate. Add 5 µL of Enzyme ("Control") and 5 µL of buffer ("Blank") wells, respectively.
2. To the Control and Blank wells, add 15 µL of the solvent in buffer that the test compounds are dissolved in. For example, if the test compounds are dissolved in buffer containing 0.1% DMSO, add 15 µL of this solution to these wells.

   To the remainder of the wells containing Enzyme, add 15 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.
3. Add 40 µL of the Substrate to all wells. Briefly tap to mix. Incubate for 30 min at room temperature, protected from light. Read the FP at $\lambda_{ex/em}$ = 485/520 nm.

CALCULATION

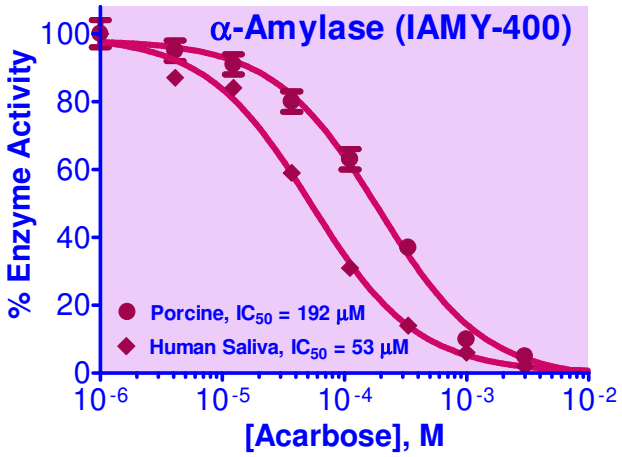
α-Amylase inhibition by a test compound is calculated as follows:

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

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

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), black, flat-bottom 384-well plates (e.g. Corning Costar), centrifuge tubes and a plate reader capable of reading FP at ($\lambda_{ex/em}$ = 485/520 nm).



Inhibition of pure porcine and human salivary α-amylase by acarbose. IC_{50} values of 192 µM and 53 µM were observed in porcine and human samples, respectively. Assays are performed in duplicate in a 384-well plate.

LITERATURE

1. Gong, L. et al. (2020). Inhibitors of α-amylase and α-glucosidase: Potential linkage for whole cereal foods on prevention of hyperglycemia. Food science & Nutrition. 8(12), 6320-6337.

2. Petrakova, L. et al. (2015). Psychosocial Stress Increases Salivary Alpha-Amylase Activity Independently from Plasma Noradrenaline Levels. PLoS ONE 10(8).

3. Sales, PM. et al. (2012). α-Amylase inhibitors: a review of raw material and isolated compounds from plant source. J Pharm Pharm Sci. 15(1): 141-83.