ab283391 – Alpha-Amylase Inhibitor Screening Kit (Colorimetric)

For the screening of Alpha-Amylase inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283391

Introduction

a-Amylases (EC 3.2.1.1) are digestive enzymes which catalyze the hydrolysis of internal a-1,4-glycosidic linkages in starch to lower molecular weight products, such glucose, maltose and maltotriose units. Human a-amylase is mainly expressed in salivary glands and pancreas. These two isozymes share a high degree of primary amino acid sequence similarity (97%) and 92% in their catalytic domains. Furthermore, both a-Amylases are immunologically identical in their reactions with polyclonal antibodies, share same mode of action, preferred substrates, are Chlorine activated and reach maximum activity at similar pH values. Functionally, they have similar but not identical cleavage patterns when tested with a variety of substrates. Modulation of a-amylase activity affects the utilization of carbohydrates as an energy source. This enzyme is responsible for the breakdown of complex carbohydrates in humans. Thus, inhibition of a-amylase could be considered as a strategy for the treatment of disorders related to carbohydrate uptake, such as diabetes, obesity, dental cavities and periodontal diseases. Alpha-Amylase Inhibitor Screening Kit (Colorimetric) (ab283391) can be used to screen/characterize potential inhibitors of Alpha-Amylase.

Storage and Stability

On receipt entire assay kit should be stored at -20°C.

Materials Supplied

Item	Quantity	Storage Condition
Amylase Assay Buffer	55 mL	-20°C
a-Amylase Inhibitor	1 vial	-20°C
a-Amylase Substrate	1 vial	-20°C
alpha-Amylase, Human Saliva	1 vial	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Amylase Assay Buffer: Warm to room temperature (RT) before use. Store at 4°C or -20°C.

a-Amylase Substrate: Reconstitute with 100 µL Amylase Assay Buffer to prepare stock solution. Aliquot Stock Solution in 10 µL aliquots and store at -20°C. Use aliquot once only.

alpha-Amylase, Human Saliva: Reconstitute with 100 µL Amylase Assay Buffer to prepare stock solution. Aliquot Stock Solution in 10 µL aliquots and store at -20°C. Use aliquot once only.

a-Amylase Inhibitor: Reconstitute in 100 uL of Amylase Assay Buffer to prepare stock solution. Aliquot Stock Solution in 10 µL aliquots and store at -20°C. Use aliquot once only. Keep on ice while in use.

Assay Protocol

Screening Compounds, Inhibitor Control & Background Control preparations:

1. Dissolve test samples 100X in a proper solvent.
2. Further dilute to 3X with Amylase Assay Buffer. Mix well.

Test Samples [S]: Add 50 µL of Diluted test samples (3X) to designated wells of a clear 96 well microplate.

Inhibitor Control [IC]: Add 10 µL of reconstituted a-Amylase Inhibitor to 40 µL Amylase Assay Buffer/Assay Buffer in designated wells

Enzyme Control [EC]: Add 50 µL of Amylase Assay Buffer to designated wells.

Background Control [BC]: Add 100 µL of Amylase Assay Buffer to designated wells.

Solvent Control [SC]: Add 50 µL of 3X Solvent (the same concentration of solvent as in the diluted Test Samples) in Amylase Assay Buffer to designated wells.

IC_{50} estimation (Optional): prepare several dilutions of candidate(s) in Amylase Assay Buffer maintaining Solvent Concentration constant for all concentrations. Add 50 µL of each dilution into designated individual wells.

Δ Note: *Various organic solvents may reduce the Alpha-Amylase enzymatic activity. Prepare parallel well(s) as Solvent Control [SC] to test the effect of the solvent on Alpha-Amylase-Amylase enzymatic activity. If [SC] slope is significantly different when compared to [EC], use [SC] values to determine effect of the respective tested compounds (see Step-Calculations).*

Alpha-Amylase Enzyme Solution Preparation:

1. Dilute Alpha-Amylase stock by adding 490 µL of Amylase Assay Buffer into 10 µL of Alpha-Amylase Enzyme.
2. Mix thoroughly by pipetting up and down.
3. Add 50 µL of diluted Alpha-Amylase Solution to each well containing Test Sample(s) [S], Inhibitor Control [IC], Enzyme Control [EC] and Solvent Control [SC].
4. Avoid introducing any bubbles into the wells.
5. Gently shake the plate to mix and incubate at room temperature (RT) for 10 minutes, protected from light.

Δ Note: *Do not store Diluted Alpha-Amylase Enzyme Solution. Discard unused solution.*

a-Amylase Substrate Mix Preparation:

1. Dilute Substrate by adding 490 µL of Amylase Assay Buffer into 10 µL of a-Amylase Substrate.
2. Mix thoroughly by pipetting up and down.
3. Add 50 µL of diluted a-Amylase Substrate to each well containing Test Sample(s) [S], Inhibitor Control [IC], Enzyme Control [EC], Background Control [BC] and Solvent Control [SC].
4. Avoid introducing any bubbles into the wells. Mix well.

Measurement:

Measure absorbance at OD = 405 nm in kinetic mode for 20-25 min at room temperature. Choose two time points (t_1 and t_2) in the linear range of the plot and obtain the corresponding values for the absorbance (OD_1 and OD_2).

Calculation:

1. Subtract the reading of Background Control [BC] from all test samples [S], Enzyme Control [EC], and Solvent Control [SC].
2. Calculate the slope of all wells by dividing the net ΔOD (OD_2 - OD_1) over time Δt (t_2 - t_1).
3. If [SC] slope is significantly different when *compared to [EC], use [SC] values to determine the inhibitory effect of tested compound(s)*.

% Relative Inhibition = $\frac{slope\ of\ [EC] - slope\ of\ [S]}{slope\ of\ [\mathrm{EC}]} \times 100$

% Relative activity = $\frac{slope\ of\ [S]}{slope\ of\ [EC]} \times 100$

Technical Support

For all technical or commercial enquiries please go to:
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