Alpha-Amylase Inhibitor Screening Kit (Colorimetric)

Cat. No. KITZ-005 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Alpha-Amylase Inhibitor Screening Kit (Colorimetric) can be used to screen/characterize potential inhibitors of Alpha-Amylase.
Description	Alpha-Amylase enzyme is responsible for the breakdown of complex carbohydrates in humans. Thus, inhibition of α-amylase could be considered as a strategy for the treatment of disorders related to carbohydrate uptake, such as obesity, diabetes, dental cavities and periodontal diseases. In this kit human Alpha-Amylase hydrolyzes the synthetic substrate, yielding smaller fragments containing the chromophore (pNP; OD = 405 nm). A potent, specific inhibitor is also included in the kit. The one-step assay is simple and can be completed within 1 hour.
Storage	On receipt entire assay kit should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.
Kit Components	Alpha-Amylase Assay Buffer 1 x 55ml Alpha-Amylase Inhibitor 1 x 100µ Alpha-Amylase Substrate 1 vial Alpha-Amylase, Human Saliva 1 vial
Materials Required but Not Supplied	96-well clear plate with flat bottom Multi-well spectrophotometer (ELISA reader)
Detection method	Colorimetric, OD = 405 nm
Assay Protocol	Reagent Preparation:

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

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

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

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

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

4.Alpha-Amylase Inhibitor: Ready to use. 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 Alpha-Amylase Assay Buffer. Mix well.

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

-Inhibitor Control [IC]: Add 10 µ of reconstituted Alpha-Amylase inhibitor to 40 µ Assay Buffer in designated wells.

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

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

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

-IC50 estimation (Optional): prepare several dilutions of candidate(s) in Alpha-Amylase Assay Buffer maintaining Solvent Concentration constant for all concentrations. Add 50 µ 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 5-Calculations).

Alpha-Amylase Enzyme Solution Preparation:

1. Dilute Alpha-Amylase stock by adding 490 µL of Alpha-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. Mix well.
5. Incubate at room temperature (RT) for 10 minutes, protected from light.

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

Alpha-Amylase Substrate Mix Preparation:

1. Dilute Substrate by adding 490 µ of Alpha-Amylase Assay Buffer into 10 µ of Alpha Amylase Substrate.
2. Mix thoroughly by pipetting up and down.
3. Add 50 µ of diluted Alpha-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 (t1 and t2) in the linear range of the plot and obtain the corresponding values for the absorbance (OD1 and OD2).

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 (OD2 - OD1) over time Δt (t2 - t1).

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

	3. If [SC] slope is significantly different when compared to [EC], use [SC] values to determine the inhibitory effect of tested compound(s).

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA