

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>

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Storage and Stability

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.

Materials Supplied

Item	Quantity	Storage Condition
Amylase Assay Buffer/Alpha-Amylase Assay Buffer	55 mL	-20°C
α -Amylase Inhibitor/Alpha-Amylase Inhibitor	100 μ L	-20°C
α -Amylase Substrate/Alpha-Amylase Substrate	1 vial	-20°C
α -Amylase, Human Saliva/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/Alpha-Amylase Assay Buffer: Warm to room temperature (RT) before use. Store at 4°C or -20°C.

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

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

α -Amylase Inhibitor/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 Amylase Assay Buffer/Alpha-Amylase Assay Buffer. Mix well.

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

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

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

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

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

IC₅₀ estimation (Optional): prepare several dilutions of candidate(s) in Amylase Assay Buffer/Alpha-Amylase Assay Buffer maintaining Solvent Concentration constant for all concentrations. Add 50 μ L 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 μ L of Amylase Assay Buffer/Alpha-Amylase Assay Buffer into 10 μ L of Alpha-Amylase Enzyme.
2. Mix thoroughly by pipetting up and down.
3. Add 50 μ L 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.

α -Amylase Substrate/Alpha-Amylase Substrate Mix Preparation:

1. Dilute Substrate by adding 490 μ L of Amylase Assay Buffer/Alpha-Amylase Assay Buffer into 10 μ L of α -Amylase Substrate/Alpha-Amylase Substrate.
2. Mix thoroughly by pipetting up and down.
3. Add 50 μ L of diluted α -Amylase Substrate/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 (t_1 and t_2) 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 ($OD_2 - OD_1$) 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).

$$\% \text{ Relative Inhibition} = \frac{\text{slope of (EC)} - \text{slope of (S)}}{\text{slope of (EC)}} \times 100$$

$$\% \text{ Relative activity} = \frac{\text{slope of (S)}}{\text{slope of (EC)}} \times 100$$

Technical Support

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