ab284520 – alpha-Glucosidase Inhibitor Screening Kit (Colorimetric)

For the screening/characterizing a-Glucosidase inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284520

Introduction

a-Glucosidase (EC 3.2.1.20) is localized in the brush border of the small intestine and is responsible for the enzymatic hydrolysis of 1,4- linked polysaccharides, producing glucose as one of the main products. Due to the vital role of glucose as one of the main sources of energy in eukaryotes, a-Glucosidase is a target for the modulation of postprandial hyperglycemia. a-Gluosidase Inhibitors (AGIs) such as Acarbose, Miglitol and Voglibose are anti-diabetic medicines that help to reduce post-meal blood glucose levels by arresting glucose absorption in the gastrointestinal tract. In addition, recent research is also focused on the discovery of natural products that could act as a-Glucosidase Inhibitors. The a-Glucosidase Inhibitor Screening Kit (ab284520) (K938) can be used to screen potential inhibitors of this enzyme. It utilizes the ability of an active a-Glucosidase to cleave a synthetic substrate thus, releasing a chromophore (OD: 410 nm). In the presence of an a-Glucosidase specific inhibitor, the enzymatic activity is greatly reduced which is detected by a decrease of absorbance readings.

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light.

Materials Supplied

Item	Quantity	Storage Condition
a-Glucosidase Assay Buffer	25 mL	-20°C
a-Glucosidase Substrate Mix	300 µL	-20°C
alpha-Glucosidase Positive Control	4 vial	-20°C
Acarbose	140 µL	-20°C

PLEASE NOTE: alpha-Glucosidase Positive Control was previously labelled as a-Glucosidase Positive Control and a-Glucosidase. The composition has not changed.

Materials Required, Not Supplied

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

- Temperature-controlled plate reader
- 96-well clear plate with flat bottom, UV transparent plate is preferred.

Reagent Preparation

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

a-Glucosidase Assay Buffer: Warm to room temperature (RT) before use. Store at -20°C.

a-Glucosidase Substrate Mix: Ready to use as supplied. If precipitate is observed, briefly sonicate contents. Store at -20°C.

alpha-Glucosidase Positive Control: Reconstitute a fresh vial with 100 µL dH_2O to prepare stock solution. This component is intended to be single use and will lose activity after reconstitution if stored. Discard excess after use.

Acarbose: Ready to use. Keep on ice while in use. Use within two months.

Assay Protocol

Screening Compounds, Inhibitor Control & Background Control preparations:

Test Samples [S] and Inhibitor Control [IC]: Dissolve test samples to 100X in a proper solvent. Further dilute to 10X using a-Glucosidase Assay Buffer. Add 10 µL of Diluted test compound, 10 µL of Acarbose into wells of 96-well clear plate designated as test samples [S] or Inhibitor Control [IC], respectively.

Enzyme Control [EC] and Background Control [BC]: Add 10 and 20 µL of a-Glucosidase Assay Buffer into designated well(s) of 96-well clear plate, respectively. IC50 estimation (Optional): prepare several dilutions of candidate(s) in a-Glucosidase Assay Buffer. Add 10 µL of each dilution into designated wells.

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

	[S]	[IC]	[EC]	[BC]	[SC]
Test Sample	10 µl	-	-	-	-
Acarbose	-	10 µl	-	-	-
α-Glucosidase Assay Buffer	-	-	10 µl	20 µl	-
Solvent Control	-	-	-	-	10 µl

- **a-Glucosidase Enzyme Solution Preparation:**
  1. Prepare a 20-fold dilution of alpha-Glucosidase Positive Control (i.e. Dilute of 2 µL of alpha-Glucosidase Positive Control with 38 µL of a-Glucosidase Assay Buffer), mix thoroughly and keep on ice.
  2. Add 10 µL of Diluted a-Glucosidase Enzyme Solution to each well containing Test Sample(s) [S], Inhibitor Control [IC], Enzyme Control [EC] and Solvent Control [SC]. Adjust the volume of each well to 80 µL/well with a-Glucosidase Assay Buffer.
  3. Mix well and incubate at room temperature for 15-20 min. Protect from light.

Δ Note: *Do not store Diluted a-Glucosidase Enzyme Solution. Discard unused solution.*

- **alpha-Glucosidase Preparation:** Mix enough reagents for the number of assays to be performed. For each well, prepare 20 µL Reaction Mix containing:

	Reaction Mix
a-Glucosidase Assay Buffer	17 µL
a-Glucosidase Substrate Mix	3 µL

- Mix & add 20 µL Reaction Mix to test sample(s) [S], Inhibitor Control [IC], Enzyme Control [EC], Solvent Control [SC] and Background Control [BC] wells and mix well.

Measurement

Measure absorbance immediately at OD: 410 nm in kinetic mode for 60 min at room temperature. Choose two time points (t1 & t2) in the linear range of the plot and obtain the corresponding values for the absorbance (OD1 and OD2).

Calculation:

1. Calculate the slope for all test samples [S], Enzyme Control [EC], Solvent Control [SC] and Background Control [BC] by dividing the net ΔOD (A2-A1) values with the time Δt (t2-t1).
2. Subtract the Slope of Background Control from [S], [EC] and [SC]. If [SC] slope is significantly different when compared to [EC], use [SC] values to determine effect of tested compound.

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

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

Technical Support

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