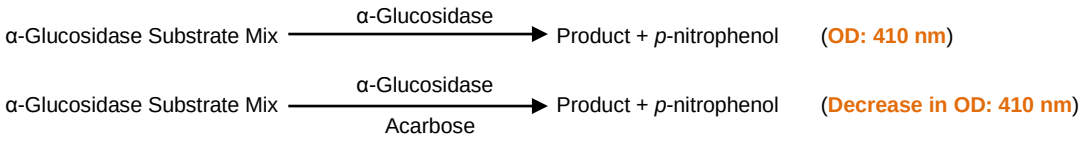
α-Glucosidase Inhibitor Screening Kit (Colorimetric)

06/18

(Catalog # K938-100; 100 assays; Store at -20°C)

I. Introduction:

α-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, α-Glucosidase is a target for the modulation of postprandial hyperglycemia. α-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 α-Glucosidase Inhibitors. BioVision's α-Glucosidase Inhibitor Screening Kit can be used to screen potential inhibitors of this enzyme. It utilizes the ability of an active α-Glucosidase to cleave a synthetic substrate thus, releasing a chromophore (OD: 410 nm). In the presence of an α-Glucosidase specific inhibitor, the enzymatic activity is greatly reduced which is detected by a decrease of absorbance readings. The assay kit provides a rapid, simple and reliable test for high-throughput screening of α-Glucosidase inhibitors.



II. Applications:

- Screening/characterizing α-Glucosidase inhibitors

III. Kit Contents:

Components	K938-100	Cap Code	Part Number
α-Glucosidase Assay Buffer	25 ml	WM	K938-100-1
α-Glucosidase Substrate Mix	300 µl	Amber	K938-100-2
α-Glucosidase	1 vial	Blue	K938-100-3
Acarbose	140 µl	Red	K938-100-4

IV. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Temperature-controlled plate reader

V. Storage Conditions and Reagent Preparation:

Store kit at -20 °C, protect from light. Briefly centrifuge small vials prior to opening.

- **α-Glucosidase Assay Buffer:** Warm to room temperature before use. Store at 4°C or -20°C.
- **α-Glucosidase Substrate Mix:** Ready to use as supplied. If precipitate is observed, briefly sonicate contents. Store at -20°C.
- **α-Glucosidase:** Reconstitute with 100 µl dH_2O to prepare stock solution. Aliquot Stock Solution in 10 µl aliquots and store at -20 °C. Use aliquot only once. Once aliquoted use within two months.
- **Acarbose:** Ready to use. Keep on ice while in use. Use within two months.

VI. α-Glucosidase Inhibitor Screening Protocol:

1. **Screening Compounds, Inhibitor Control & Background Control preparations: Samples [S]** and **Inhibitor Control [IC]:** Dissolve test samples to 100X in a proper solvent. Further dilute to 10X using α-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 α-Glucosidase Assay Buffer into designated well(s) of 96-well clear plate, respectively. **IC_{50} estimation (Optional)**: prepare several dilutions of candidate(s) in α-Glucosidase Assay Buffer. Add 10 µl of each dilution into designated wells.

   Note: Various organic solvents may reduce the α-Glucosidase enzymatic activity. Prepare parallel well(s) as Solvent Control [SC] to test the effect of the solvent on α-Glucosidase activity. If [SC] slope is significantly different when compared to EC, use [SC] values to determine effect of the respective tested compound (see Step 5).

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

2. **α-Glucosidase Enzyme Solution Preparation:** Prepare a 20-fold dilution of α-Glucosidase (i.e. Dilute of 2 µl of α-Glucosidase with 38 µl of α-Glucosidase Assay Buffer), mix thoroughly and keep on ice. Add 10 µl of **Diluted α-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 α-Glucosidase Assay Buffer. **Mix well and incubate at room temperature for 15-20 min. Protect from light.**

   Note: Do not store Diluted α-Glucosidase Enzyme Solution. Discard unused solution.

3. **Reaction Mix Preparation:** Mix enough reagents for the number of assays to be performed. For each well, prepare 20 µl Reaction Mix containing:

	Reaction Mix
α-Glucosidase Assay Buffer	17 µl
α-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.

4. **Measurement:** Measure absorbance immediately at OD: 410 nm in kinetic mode for 60 min at room temperature. Choose two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding values for the absorbance (OD_1 and OD_2).

5. **Calculation:** Calculate the slope for all test samples [S], Enzyme Control [EC], Solvent Control [SC] and Background Control [BC] by dividing the net ΔOD (A_2-A_1) values with the time Δt (t_2-t_1). 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.

$$\% \text{ Relative Inhibition} = \frac{\text{Slope of}[\text{EC}] - \text{Slope of}[\text{S}]}{\text{Slope of}[\text{EC}]} \text{X100}$$

$$\% \text{ Relative Activity} = \frac{\text{Slope of}[\text{S}]}{\text{Slope of}[\text{EC}]} \text{X100}$$

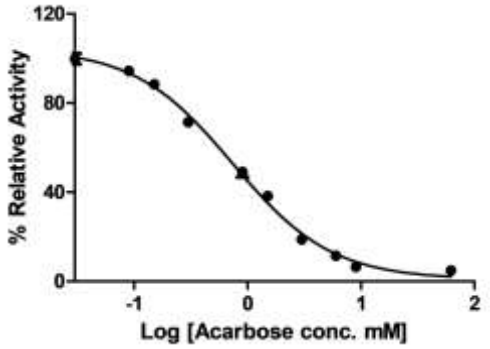


Figure: Inhibition of α-Glucosidase activity by Acarbose. IC_{50} of Acarbose was calculated to be 0.74 ± 0.15 mM. Assay was carried out following the kit protocol.

VII. RELATED PRODUCTS:

α-Glucosidase Activity Colorimetric Assay Kit (K690)
Starch Colorimetric/Fluorometric Assay Kit (K647)
Glucose and Sucrose Colorimetric/Fluorometric Assay Kit (K616)
Glucose Colorimetric Assay Kit II (K686)
Glucose-6-phosphate Dehydrogenase Assay Kit (K757)
Glucose Uptake Colorimetric Assay Kit (K676)
Glycogen Colorimetric/Fluorometric Assay Kit (K646)
Hexokinase Colorimetric Assay Kit (K789)
Maltose Colorimetric/Fluorometric Assay Kit (K628)
Total Carbohydrate Assay Kit (K645)
Amylase Activity Colorimetric Assay Kit (K711)
Glucose Colorimetric/Fluorometric Assay Kit (K666)
PicoProbe™ Glucose Fluorometric Assay Kit (K688)
Glucose Dehydrogenase Activity Assay Kit (K786)
PicoProbe™ Glucose-6-Phosphate Fluorometric Assay Kit (K687)
Glucose Uptake Fluorometric Assay Kit (K666)
Glycogen Colorimetric Assay Kit II (K648)
PicoProbe™ Glucokinase Activity Assay Kit (K969)
Maltose & Glucose Colorimetric/Fluorometric Assay Kit (K618)
PicoProbeTM Glucose-6-Phosphate Assay Kit (K687)

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