# alpha-Glucosidase Inhibitor Screening Kit (Colorimetric)

**Cat. No.** KITZ-006 **Lot. No.** (See product label)

## SPECIFICATION

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| **Product Overview** | The assay kit provides a rapid, simple and reliable test for high-throughput screening of α-Glucosidase inhibitors. |
| **Description** | alpha-Glucosidase Inhibitor Screening Kit (Colorimetric) 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. |
| **Storage** | On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted. |
| **Kit Components** | α-Glucosidase Assay Buffer 25 mL<br>α-Glucosidase Substrate Mix 300 mL<br>α-Glucosidase 1 vial<br>Acarbose 140 µ |
| **Materials Required but Not Supplied** | Temperature-controlled plate reader<br>96-well clear plate with flat bottom, UV transparent plate is preferred |
| **Detection method** | Colorimetric, OD = 410 nm |
| **Assay Protocol** | Reagent Preparation: |

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

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

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

3. α-Glucosidase: Reconstitute with 100 µ dH2O to prepare stock solution. Aliquot Stock Solution in 10 µ aliquots and store at -20°C. Use aliquot only once. Once aliquoted use within two months.

4. 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 α-Glucosidase Assay Buffer. Add 10 µ of Diluted test compound, 10 µ 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 µ of α-Glucosidase Assay Buffer into designated well(s) of 96-well clear plate, respectively.

IC50 estimation (Optional): prepare several dilutions of candidate(s) in α-Glucosidase Assay Buffer. Add 10 µ of each dilution into designated wells.

Δ 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 Calculation section).

α-Glucosidase Enzyme Solution Preparation:

1.Prepare a 20-fold dilution of α-Glucosidase (i.e. Dilute of 2 µ of α-Glucosidase with 38 µ of α-Glucosidase Assay Buffer), mix thoroughly and keep on ice.

2.Add 10 µ of Diluted α-Glucosidase Enzyme Solution to each well containing Test Sample(s) [S], Inhibitor Control [IC], Enzyme Control [EC] and Solvent Control [SC].

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Adjust the volume of each well to 80 µ/well with α-Glucosidase Assay Buffer.
3.Mix well and incubate at room temperature for 15-20 min. Protect from light.
Δ Note: Do not store Diluted α-Glucosidase Enzyme Solution. Discard unused solution.
Aldose Reductase Substrate Preparation: Mix enough reagents for the number of assays to be performed. For each well, prepare 20 µ Reaction Mix containing:

Mix & add 20 µ 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.

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