α-Glucosidase Inhibitor Screening Kit (Colorimetric)  
(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-Glucosidase 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.

![Diagram of α-Glucosidase reaction]

α-Glucosidase Substrate Mix → α-Glucosidase → Product + p-nitrophenol (OD: 410 nm)

α-Glucosidase Substrate Mix → Acarbose → Product + p-nitrophenol (Decrease in OD: 410 nm)

II. Applications:
• Screening/characterizing α-Glucosidase inhibitors

III. Kit Contents:

<table>
<thead>
<tr>
<th>Components</th>
<th>K938-100</th>
<th>Cap Code</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Glucosidase Assay Buffer</td>
<td>25 ml</td>
<td>WM</td>
<td>K938-100-1</td>
</tr>
<tr>
<td>α-Glucosidase Substrate Mix</td>
<td>300 µl</td>
<td>Amber</td>
<td>K938-100-2</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>1 vial</td>
<td>Blue</td>
<td>K938-100-3</td>
</tr>
<tr>
<td>Acarbose</td>
<td>140 µl</td>
<td>Red</td>
<td>K938-100-4</td>
</tr>
</tbody>
</table>

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.
• Acarbose: Reconstitute with 100 µl dH2O 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.

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. IC50 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).

<table>
<thead>
<tr>
<th></th>
<th>[S]</th>
<th>[IC]</th>
<th>[EC]</th>
<th>[BC]</th>
<th>[SC]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Sample</td>
<td>10 µl</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Acarbose</td>
<td>−</td>
<td>10 µl</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>α-Glucosidase Assay Buffer</td>
<td>−</td>
<td>−</td>
<td>10 µl</td>
<td>20 µl</td>
<td>−</td>
</tr>
<tr>
<td>Solvent Control</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>10 µl</td>
<td>−</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Reaction Mix</th>
<th>µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Glucosidase Assay Buffer</td>
<td>17</td>
</tr>
<tr>
<td>α-Glucosidase Substrate Mix</td>
<td>3</td>
</tr>
</tbody>
</table>

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 (t₁ & t₂) in the linear range of the plot and obtain the corresponding values for the absorbance (OD₁ and OD₂).

5. Calculation: Calculate the slope for all test samples [S], Enzyme Control [EC], Solvent Control [SC] and Background Control [BC] by dividing the net ∆OD (A₂-A₁) values with the time ∆t (t₂-t₁). 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{\text{Slope of [EC]} - \text{Slope of [S]}}{\text{Slope of [EC]}} \times 100

% Relative Activity = \frac{\text{Slope of [S]}}{\text{Slope of [EC]}} \times 100

Figure: Inhibition of α-Glucosidase activity by Acarbose. IC₅₀ 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)
- Amylase Activity Colorimetric Assay Kit (K711)
- Starch Colorimetric/Fluorometric Assay Kit (K647)
- Glucose Colorimetric/Fluorometric Assay Kit (K666)
- Glucose and Sucrose Colorimetric/Fluorometric Assay Kit (K616)
- PicoProbe™ Glucose Fluorometric Assay Kit (K688)
- Glucose Colorimetric Assay Kit II (K686)
- Glucose Dehydrogenase Activity Assay Kit (K786)
- Glucose-6-phosphate Dehydrogenase Assay Kit (K757)
- PicoProbe™ Glucose-6-Phosphate Fluorometric Assay Kit (K687)
- Glucose Uptake Colorimetric Assay Kit (K676)
- Glucose Uptake Fluorometric Assay Kit (K666)
- Glycogen Colorimetric/Fluorometric Assay Kit (K646)
- Glycogen Colorimetric Assay Kit II (K648)
- Hexokinase Colorimetric Assay Kit (K789)
- PicoProbe™ Glucokinase Activity Assay Kit (K969)
- Maltose Colorimetric/Fluorometric Assay Kit (K628)
- Maltose & Glucose Colorimetric/Fluorometric Assay Kit (K618)
- Total Carbohydrate Assay Kit (K645)
- PicoProbeTM Glucose-6-Phosphate Assay Kit (K687)

FOR RESEARCH USE ONLY! Not to be used on humans.