**DESCRIPTION**

Glucose oxidase catalyzes the oxidation of glucose from D-glucose to D-glucono-δ-lactone. Physiologically, it aids in the breakdown of glucose into smaller metabolites. It is widely used in electrochemical glucose sensors designed for diabetes patients. Simple, direct and high-throughput assays for measuring glucose oxidase activity are described in the Material Safety Data Sheet. BioAssay Systems' glucose oxidase assay kit uses a single Working Reagent that combines the glucose oxidase reaction and color reaction in one step. The change in color intensity of the reaction product at 570 nm or fluorescence intensity at λ<sub>systm</sub> = 530/585 nm is directly proportional to glucose oxidase activity in the sample.

**KEY FEATURES**

Sensitive and accurate. Use as little as 20 µL samples. Linear detection range in 96-well plate for 20 minute incubation at 25°C: 0.02 to 10 U/L glucose oxidase for colorimetric assays and 0.002 to 1.5 U/L for fluorimetric assays.

Simple and high-throughput. The procedure involves addition of a single working reagent and incubation for 20 min at room temperature.

**APPLICATIONS**

Direct Assays: glucose oxidase activity in cell lysate, culture medium and other biological samples.

Drug Discovery/Pharmacology: effects of drugs on glucose metabolism.

**KIT CONTENTS (100 tests in 96-well plates)**

- **Assay Buffer:** 10 mL
- **HRP Enzyme:** 120 µL
- **Glucose:** 1.5 mL 2 M Glucose
- **Dye Reagent:** 120 µL
- **Standard:** 100 µL 3% H<sub>2</sub>O<sub>2</sub>
- **Storage conditions:** The kit is shipped on ice. Store all components at -20°C. Shelf life: 6 months after receipt.

**Precautions:** Reagents are for research use only. Normal precautions for laboratory reagents should be followed while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

**COLORIMETRIC PROCEDURE**

Samples can be analyzed immediately after collection, or stored in aliquots at -20°C. Avoid repeated freeze-thaw cycles. If particulates are present, centrifuge sample and use clear supernatant for assay.

1. Equilibrate all components to room temperature. During experiment, keep thawed Enzyme in a refrigerator or on ice.

2. **H<sub>2</sub>O<sub>2</sub> Standard Curve.** Mix 5 µL 3% H<sub>2</sub>O<sub>2</sub> and 914 µL dH<sub>2</sub>O (final 4.8 mM) then mix 20 µL of the 4.8 mM H<sub>2</sub>O<sub>2</sub> with 460 µL dH<sub>2</sub>O to yield 200 µM H<sub>2</sub>O<sub>2</sub>. Prepare standards as shown in the Table below.

<table>
<thead>
<tr>
<th>No</th>
<th>400 µM H&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt; + H&lt;sub&gt;2&lt;/sub&gt;O</th>
<th>Vol (µL)</th>
<th>H&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt; (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100 µL + 0 µL</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>2</td>
<td>60 µL + 40 µL</td>
<td>100</td>
<td>120</td>
</tr>
<tr>
<td>3</td>
<td>30 µL + 70 µL</td>
<td>100</td>
<td>60</td>
</tr>
<tr>
<td>4</td>
<td>0 µL + 100 µL</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Transfer 20 µL standards and samples into separate wells.

3. **Working Reagent.** Prepare bulk working reagent by mixing 75 µL Assay Buffer, 10 µL 2 M Glucose, 1 µL HRP Enzyme (vortex briefly before pipetting), and 1 µL Dye Reagent per reaction well in a clean tube. Transfer 80 µL Working Reagent into each reaction well. Tap plate to mix.

4. Read optical density immediately (OD<sub>ex</sub>) at 570 nm (550-585 nm). Incubate 20 min at room temperature, and then read optical density again (OD<sub>ex</sub>).

**FLUORIMETRIC PROCEDURE**

For fluorimetric assays, the linear detection range is 0.002 to 1.5 U/L glucose oxidase. Dilute the standards from Colorimetric Procedure 10× with dH<sub>2</sub>O to obtain standards at 20, 12, 6 and 0 µM H<sub>2</sub>O<sub>2</sub>. Transfer 20 µL standards and 20 µL samples into separate wells of a black 96-well plate.

Add 80 µL Working Reagent (see Colorimetric Procedure), tap plate to mix.

Read fluorescence immediately (F<sub>i</sub>) at λ<sub>systm</sub> = 530/585 nm, incubate 20 min at room temperature, and then read fluorescence again (F<sub>f</sub>).

**CALCULATION**

Subtract blank OD<sub>ex</sub> or F<sub>ex</sub> (water, #4) from all standard OD<sub>ex</sub> or F<sub>ex</sub> values and plot the ΔOD or ΔF against standard concentrations. Determine the slope using linear regression. Calculate the ΔOD<sub>Sample</sub> or ΔF<sub>Sample</sub> of all samples by subtracting OD<sub>ex</sub> or F<sub>ex</sub> from OD<sub>ex</sub> or F<sub>ex</sub> for each sample. Do the same for the blank (water, standard #4) to get ΔOD<sub>Blank</sub> or ΔF<sub>Blank</sub>. Calculate the activity using the equation below:

\[
\text{GO Activity} = \frac{\Delta F_{\text{Sample}} - \Delta F_{\text{Blank}}}{\text{Slope} (\mu M^{-1} \times m)} \times n \ (U/L)
\]

Where ΔF<sub>Sample</sub> and ΔF<sub>Blank</sub> are the change in optical density or fluorescent values of the sample and blank, respectively. Slope is the slope of the H<sub>2</sub>O<sub>2</sub> standard curve, t is the incubation time (20 minutes), and n is the dilution factor.

**Notes:** If the calculated sample glucose concentration is higher than 10 U/L in colorimetric assay or 1.5 U/L in fluorimetric assay, dilute sample in water and repeat the assay. Multiply result by the dilution factor (n). For samples with low Glucose Oxidase activity, the incubation time can be increased.

**Unit definition:** 1 U/L of Glucose Oxidase catalyzes 1 µmole of H<sub>2</sub>O<sub>2</sub> per minute at pH 7.0 and room temperature.

**MATERIALS REQUIRED, BUT NOT PROVIDED**

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates, black 96-well plates and plate reader.

**LITERATURE**