**EnzyChrom™ Glycogen Assay Kit (Cat# E2GN-100)**
Quantitative Colorimetric/Fluorimetric Glycogen Determination

**DESCRIPTION**

GLYCOGEN is a branched polysaccharide of glucose units linked by α-1,4 glycosidic bonds and α-1,6 glycosidic bonds. It is stored primarily in the liver and muscle, and forms an energy reserve that can be quickly mobilized to meet a sudden need for glucose. The most common glycogen metabolism disorder is found in diabetes, in which, due to abnormal amounts of insulin, liver glycogen can be abnormally accumulated or depleted. Genetic glycogen storage diseases have been associated with various inborn errors of metabolism caused by deficiencies of enzymes necessary for glycogen synthesis or breakdown.

Simple, direct and automation-ready procedures for measuring glycogen concentrations find wide applications in research and drug discovery. BioAssay Systems’ glycogen assay uses a single Working Reagent that combines the enzymatic break down of glycogen and the detection of glucose in one step. The color intensity of the reaction product at 570nm or fluorescence intensity at λex/em = 530/585 nm is directly proportional to the glycogen concentration in the sample. This simple convenient assay is carried out at room temperature and takes only 30 min.

**KEY FEATURES**

Use as little as 10 µL samples. Linear detection range: 2 to 200 µg/mL glycogen for colorimetric assays and 0.2 to 20 µg/mL for fluorimetric assays.

**KIT CONTENTS (100 TESTS IN 96-WELL PLATES)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer</td>
<td>12 mL</td>
</tr>
<tr>
<td>Dye Reagent</td>
<td>120 µL</td>
</tr>
<tr>
<td>Enzyme A (Dried)</td>
<td>50 µL 50 mg/mL</td>
</tr>
<tr>
<td>Enzyme B</td>
<td>120 µL</td>
</tr>
</tbody>
</table>

**PROCEDURES**

**Reagent Preparation:**
Reconstitute Enzyme A by adding 120 µL Assay Buffer to the Enzyme A tube. Make sure Enzyme A is fully dissolved by pipetting up and down. Store reconstituted Enzyme A at -20°C and use within 1 month.

**Sample Preparation:**
Samples can be prepared according to Murat & Serfaty (Clin Chem. 20:1576-1577, 1974). Briefly, homogenize tissue/cell sample in 25 mM citrate, pH 4.2, 2.5 g/L NaF on ice. Centrifuge 14,000 g for 5 min to remove debris, and use 10 µL clear supernatant for the assay.

**Colorimetric Procedure:**

1. Equilibrate all components to room temperature. During experiment, keep thawed enzymes in a refrigerator or on ice.
2. Standards and samples: Dilute standard by mixing 5 µL Standard with 1,245 mL dH2O to give 200 µg/mL standard. Dilute standard in dH2O as follows:
<table>
<thead>
<tr>
<th>No</th>
<th>200 µg/mL STD + H2O (µL)</th>
<th>Vol (µL)</th>
<th>Glycogen (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200 µL + 0 µL</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>2</td>
<td>150 µL + 50 µL</td>
<td>200</td>
<td>150</td>
</tr>
<tr>
<td>3</td>
<td>100 µL + 100 µL</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>50 µL + 150 µL</td>
<td>200</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>0 µL + 200 µL</td>
<td>200</td>
<td>0</td>
</tr>
</tbody>
</table>
3. **Working Reagent.** For each reaction well, mix 90 µL Assay Buffer, 1 µL Enzyme A, 1 µL Enzyme B and 1 µL Dye Reagent in a clean tube. Transfer 90 µL Working Reagent into each reaction well. Tap plate to mix.
4. Incubate 30 min at room temperature. Read optical density at 570 nm (550-585 nm).

**Fluorimetric Procedure:**

For fluorimetric assays, the linear detection range is 0.2 to 20 µg/mL glycogen. Follow steps 1-3 of the colorimetric procedure, but prepare 0, 5, 10, 15 and 20 µg/mL Standard and use a black flat-bottom microplate. Incubate 30 min at room temperature and read fluorescence at λex = 530 nm and λem = 585 nm.

**RESULTS**

Subtract Blank reading (OD570nm or fluorescence intensity) from the standard reading values and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the glycogen concentration of the sample.

**CALCULATION**

\[
\text{Glycogen} = \frac{R_{\text{Sample}} - R_{\text{Blank}}}{\text{Slope}} \times \mu g/mL
\]

R<sub>Sample</sub> and R<sub>Blank</sub> are the OD<sub>570nm</sub> or fluorescence intensity values of the sample and blank (water, or sample blank, see below).

**GENERAL CONSIDERATIONS**

1. Prepare Sample Blank reagent by mixing 90 µL Assay Buffer, 1 µL Enzyme B, and 1 µL Dye Reagent (No Enzyme A). Add this reagent only to the Sample Blank wells. Subtract the OD or fluorescence of the Sample Blank from the sample readings to calculate glycogen concentration.
2. This assay is based on a kinetic reaction, the use of a multi-channel pipettor for adding the working reagent is recommended.
3. Interference. SH-group containing reagents (e.g., DTT, β-mercaptoethanol) may interfere with this assay and should be avoided in sample preparation.

**MATERIALS REQUIRED, BUT NOT PROVIDED**

Pipetting devices, centrifuge tubes, clear flat-bottom uncoated 96-well plates (e.g. VWR cat# 82050-760), optical density plate reader; black flat-bottom uncoated 96-well plates (e.g. VWR cat# 82050-676), fluorescence plate reader.

**PUBLICATIONS**