**EnzyChrom™ Glycerol Assay Kit (Cat# EGLY-200)**

**Quantitative Colorimetric/Fluorimetric Glycerol Determination**

**DESCRIPTION**

GLYCEROL (GLYCERIN or GLYCERINE, C₃H₅(OH)₂) is widely used in foods, beverages and pharmaceutical formulations. It is also a main by-product of biodiesel production. Simple, direct and automation-ready procedures for measuring glycerol concentrations find wide applications. BioAssay glycerol assay uses a single Working Reagent that combines glycerol kinase, glycerol phosphate oxidase and color reactions in one step. The color intensity of the reaction product at 570nm or fluorescence intensity at λem/ex = 585/530nm is directly proportional to glycerol concentration in the sample.

**KEY FEATURES**

- **Sensitive and accurate.** Use as little as 10 µL samples. Linear detection range in 96-well plate: 10 to 1000 µM (92 µg/dL to 9.2 mg/dL) glycerol for colorimetric assays and 2 to 50 µM for fluorimetric assays.
- **Simple and convenient.** The procedure involves addition of a single working reagent and incubation for 20 min at room temperature, compatible for HTS assays.
- **Improved reagent stability.** The optimized formulation has greatly enhanced the reagent and signal stability.

**APPLICATIONS:**

- **Direct Assays:** glycerol in biological samples (e.g. serum and plasma).
- **Drug Discovery/Pharmacology:** effects of drugs on glycerol metabolism.
- **Food and Beverages:** glycerol in food, beverages, pharmaceutical formulations etc.

**KIT CONTENTS**

- **Assay Buffer:** 24 mL
- **Enzyme Mix:** 500 µL ATP: 250 µL
- **Dye Reagent:** 0.22 mL Standard: 100 µL 100 mM Glycerol

**Storage conditions.** The kit is shipped on dry ice. Store Assay Buffer at 4°C and other reagents at -20°C. Shelf life of three months after receipt.

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

**COLORIMETRIC PROCEDURE**

Note: SH-group containing reagents (e.g. mercaptoethanol, DTT) may interfere with this assay and should be avoided in sample preparation.

1. Equilibrate all components to room temperature. Keep thawed Enzyme Mix in a refrigerator or on ice. Dilute standard in distilled water as follows (diluted standards can be used for future assays when stored refrigerated).

<table>
<thead>
<tr>
<th>No</th>
<th>STD + H₂O (µL)</th>
<th>Vol (µL)</th>
<th>Glycerol (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 + 990</td>
<td>1000</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>6 + 994</td>
<td>1000</td>
<td>0.6</td>
</tr>
<tr>
<td>3</td>
<td>3 + 997</td>
<td>1000</td>
<td>0.3</td>
</tr>
<tr>
<td>4</td>
<td>0 + 1000</td>
<td>1000</td>
<td>0</td>
</tr>
</tbody>
</table>

Transfer 10 µL standards and 10 µL samples into separate wells of a clear 96-well plate.

2. For each reaction well, mix 100 µL Assay Buffer, 2 µL Enzyme Mix, 1 µL ATP and 1 µL Dye Reagent in a clean tube. This Working Reagent should be used on the same day of preparation. Transfer 100 µL Working Reagent into each reaction well. Tap plate to mix.

3. Incubate 20 min at room temperature. Read optical density at 570nm (550-585nm).

**FLUORIMETRIC PROCEDURE**

For fluorimetric assays, the linear detection range is 2 to 50 µM glycerol. Mix 10 µL 100 mM Standard with 990 µL H₂O (final 1 mM).

<table>
<thead>
<tr>
<th>No</th>
<th>1 mM STD + H₂O</th>
<th>Vol (µL)</th>
<th>Glycerol (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50 µL + 950 µL</td>
<td>1000</td>
<td>0.60</td>
</tr>
<tr>
<td>2</td>
<td>100 µL + 900 µL</td>
<td>1000</td>
<td>0.30</td>
</tr>
<tr>
<td>3</td>
<td>15 µL + 895 µL</td>
<td>1000</td>
<td>0.015</td>
</tr>
<tr>
<td>4</td>
<td>0 µL + 1000 µL</td>
<td>1000</td>
<td>0</td>
</tr>
</tbody>
</table>

Dilute standards as above. Transfer 10 µL standards and 10 µL samples into separate wells of a black 96-well plate.

Add 100 µL Working Reagent (see Colorimetric Procedure). Tap plate to mix.

Incubate 20 min at room temperature and read fluorescence at λem = 530nm and λex = 585nm.

The glycerol concentration of Sample is calculated as

\[ [\text{Glycerol}] = \frac{[\text{Glycerol}]_{\text{Fsample}} - [\text{Glycerol}]_{\text{Fblank}}}{\text{Slope}} \] (mM)

**LITERATURE**


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