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

Quantitative Colorimetric/Fluorimetric Glycerol Determination

DESCRIPTION

GLYCEROL [*GLYCERIN* or *GLYCERINE*, $C_3H_5(OH)_3$] 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: 220 µL **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.

COLORIEMTRIC 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).

No	STD + H_2O	Vol (µL)	Glycerol (mM)
1	10 µL + 990 µL	1000	1.0
2	6 µL + 994 µL	1000	0.6
3	3 µL + 997 µL	1000	0.3
4	0 µL + 1000 µL	1000	0

   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).

Note: if the Sample OD is higher than the Standard OD at 1.0 mM, dilute sample in water and repeat the assay. Multiply result by the dilution factor.

CALCULATION

Subtract blank OD (water, #4) from the standard OD values and plot the OD against standard concentrations. Determine the slope using linear regression fitting. The glycerol concentration of Sample is calculated as

$$[\text{Glycerol}] = \frac{OD_{SAMPLE} - OD_{H2O}}{\text{Slope}} \quad (\text{mM})$$

OD_{SAMPLE} and OD_{H2O} are optical density values of the sample and water. **Conversions**: 1mM glycerol equals 9.2 mg/dL, 92 ppm.

FLUORIMETRIC PROCEDURE

For fluorimetric assays, the linear detection range is 2 to 50 µM glycerol. Mix 10 µL 100 mM Standard with 990 µL H_2O (*final* 1 mM).

No	1 mM STD + H_2O	Vol (µL)	Glycerol (mM)
1	50 µL + 950 µL	1000	0.050
2	30 µL + 970 µL	1000	0.030
3	15 µL + 985 µL	1000	0.015
4	0 µL +1000 µL	1000	0

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 λ_{ex} = 530nm and λ_{em} = 585nm.

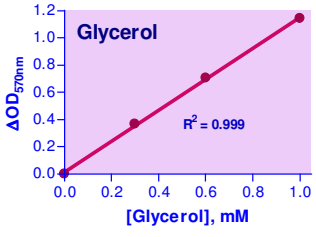
The glycerol concentration of Sample is calculated as

$$[\text{Glycerol}] = \frac{F_{SAMPLE} - F_{H2O}}{\text{Slope}} \quad (\text{mM})$$

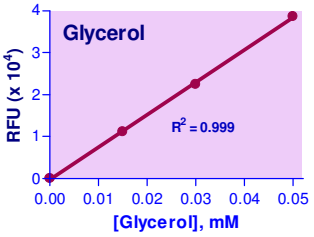
MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices, centrifuge tubes, Clear flat-bottom 96-well plates, black 96-well plates (e.g. Corning Costar) and plate reader.

Glycerol Standard Curves



96-well colorimetric assay



96-well fluorimetric assay

LITERATURE

1. Duncan RE, et al. (2007). Regulation of lipolysis in adipocytes. Annu Rev Nutr. 27:79-101.

2. Moller F, Roomi MW. (1974). An enzymatic, spectrophotometric glycerol assay with increased basic sensitivity. Anal Biochem. 59(1):248-58.

3. MacRae AR. (1977). A semi-automated enzymatic assay for free glycerol and triglycerides in serum or plasma. Clin Biochem. 10(1):16-9.

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