CREATIVE BIOMART®
Assay Kit

Free Glycerol Assay Kit

Product Information

Common Name

Glycerol

Cat.No. Kit-0377

Product Overview

Free Glycerol Assay Kit 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.

Description

GLYCEROL [GLYCERIN or GLYCERINE, C3H5(OH)3] is widely used in foods, beverages and pharmaceutical formulations. It is also a main byproduct of biodiesel production. Simple, direct and automation-ready procedures for measuring glycerol concentrations find wide applications.

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.

Usage

For research use only (RUO)

Storage

The kit is shipped on ice. Store all components at -20°C. Shelf life of 12 months after receipt.

Kit Components

Assay Buffer: 24 mL
Enzyme Mix: 500 µL
ATP: 250 µL
Dye Reagent: 220 µL

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Standard: 100 µL 100 mM Glycerol

Materials Required but Not Supplied

Pipeting devices, centrifuge tubes, Clear flat-bottom 96-well plates, black 96-well plates and plate reader.

Detection method Colorimetric, Fluorometric

Features & Benefits

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.

Assay Protocol

COLORIEMTRIC 96-WELL 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 + H2O 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.

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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
[Glycerol] = (ODSAMPLE – ODH2O)/ Slope (mM)
ODSAMPLE and ODH2O are optical density values of the sample and water.
Conversions: 1mM glycerol equals 9.2 mg/dL, 92 ppm.
FLUORIMETRIC 96-WELL 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).
No 1 mM STD + H2O 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 λex = 530nm and λem = 585nm.
The glycerol concentration of Sample is calculated as
[Glycerol] = (FSAMPLE – FH2O)/ Slope (mM)