

Free Glycerol Assay Kit

Cat. No. Kit-0377 **Lot. No.** (See product label)

SPECIFICATION

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 $\lambda_{em}/\lambda_{ex}$ = 585/530nm is directly proportional to glycerol concentration in the sample.
Description	GLYCEROL [GLYCERIN or GLYCERINE, $C_3H_5(OH)_3$] 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 μ ATP: 250 μ Dye Reagent: 220 μ Standard: 100 μ 100 mM Glycerol

 Tel: 1-631-559-9269 1-516-512-3133

 Email: info@creative-biomart.com  Fax: 1-631-938-8127

 45-1 Ramsey Road, Shirley, NY 11967, USA

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 μ 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). <table><tr><th>No</th><th>STD</th><th>H2O Vol (μ)</th><th>Glycerol (mM)</th></tr><tr><td>1</td><td>10 μ</td><td>+ 990 μ</td><td>1000 1.0</td></tr><tr><td>2</td><td>6 μ</td><td>+ 994 μ</td><td>1000 0.6</td></tr><tr><td>3</td><td>3 μ</td><td>+ 997 μ</td><td>1000 0.3</td></tr><tr><td>4</td><td>0 μ</td><td>+ 1000 μ</td><td>1000 0</td></tr></table> Transfer 10 μ standards and 10 μ samples into separate wells of a clear 96-well plate. 2. For each reaction well, mix 100 μ Assay Buffer, 2 μ Enzyme Mix, 1 μ ATP and 1 μ Dye Reagent in a clean tube. This Working Reagent should be used on the same day of preparation. Transfer 100 μ Working Reagent into each reaction well. Tap plate to mix.	No	STD	H2O Vol (μ)	Glycerol (mM)	1	10 μ	+ 990 μ	1000 1.0	2	6 μ	+ 994 μ	1000 0.6	3	3 μ	+ 997 μ	1000 0.3	4	0 μ	+ 1000 μ	1000 0
No	STD	H2O Vol (μ)	Glycerol (mM)																		
1	10 μ	+ 990 μ	1000 1.0																		
2	6 μ	+ 994 μ	1000 0.6																		
3	3 μ	+ 997 μ	1000 0.3																		
4	0 μ	+ 1000 μ	1000 0																		

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

$$[\text{Glycerol}] = (\text{ODSAMPLE} - \text{ODH}_2\text{O}) / \text{Slope (mM)}$$

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 μ M glycerol. Mix 10 μ 100 mM Standard with 990 μ H₂O (final 1 mM).

No 1 mM STD + H₂O Vol (μ) Glycerol (mM)

1 50 μ + 950 μ 1000 0.050

2 30 μ + 970 μ 1000 0.030

3 15 μ + 985 μ 1000 0.015

4 0 μ + 1000 μ 1000 0

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

Add 100 μ 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

$$[\text{Glycerol}] = (\text{FSAMPLE} - \text{FH}_2\text{O}) / \text{Slope (mM)}$$