

Free Glycerol Assay Kit

(Catalog #K630-100; 100 assays; Store at -20°C; Lot#: 40630)

I. Introduction:

Glycerol is widely used in foods, beverages, solvents, pharmaceutical and cosmetic products, etc. There is broad interest in quantification of glycerol for research and development. BioVision's Glycerol Assay Kit provides a sensitive, easy assay to measure free glycerol concentration in various samples. In the assay, glycerol is enzymatically oxidized to generate a product which reacts with the probe to generate color ($\lambda = 570$ nm) and fluorescence (Ex/Em = 535/587 nm). The kit can detect 50 pmol-10 nmol (or 1-10000 μ M range) of glycerol in various samples.

II. Kit Contents:

Components	K630-100	Cap Code	Part Number
Glycerol Assay Buffer	25 ml	WM	K630-100-1
Glycerol Probe (lyophilized)	1 vial	Red	K630-100-2
Dimethylsulfoxide (DMSO, Anhydrous)	0.4 ml	Brown	K630-100-3
Glycerol Enzyme Mix (lyophilized)	1 vial	Green	K630-100-4
Glycerol Standard (100 mM)	0.2 ml	Yellow	K630-100-5

III. Storage and Handling:

Store kit at -20°C, protect from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening.

IV. Reagents Preparation:

Glycerol Probe: Dissolve in 220 μ l anhydrous DMSO (provided) before use. Store at -20°C, protect from light and moisture. Use within two months.

Glycerol Enzyme Mix: Dissolve in 220 μ l Assay Buffer. Aliquot and store at -20°C. Use within two months.

V. Glycerol Assay Protocol:

1. Standard Curve Preparation:

For the colorimetric assay, add 10 μ l of the glycerol standard to 990 μ l of Assay Buffer to generate 1 mM glycerol standard, mix well. Add 0, 2, 4, 6, 8, 10 μ l into each well individually. Adjust volume to 50 μ l/well with Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of glycerol Standard.

For the fluorometric assay, dilute the Glycerol Standard to 0.01- 0.1 mM with the Assay Buffer (Detection sensitivity is 10-100 fold higher for a fluorometric than a colorimetric assay). Follow the protocol as for the colorimetric assay.

2. Sample Preparation: Prepare test samples to a final volume of 50 μ l/well with Assay Buffer in a 96-well plate. We suggest testing several dilutions of your sample to make sure the readings are within the standard curve range.

3. Reaction Mix: Mix enough reagent for the number of samples and standards to be performed: For each well, prepare a total 50 μ l Reaction Mix:

- 46 μ l Assay Buffer
- 2 μ l Glycerol Probe
- 2 μ l Glycerol Enzyme Mix

4. Add 50 μ l of the Reaction Mix to each well containing standard and samples. Mix well. Incubate at room temperature for 30 minutes, protect from light.

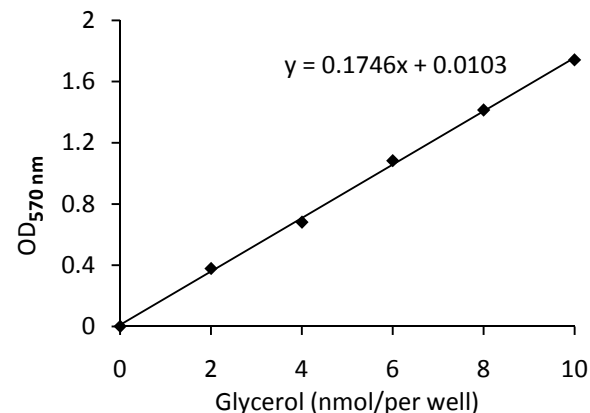
5. Measure O.D. 570 nm for the colorimetric assay or Ex/Em = 535/590 nm for the fluorometric assay in a microtiter plate reader. The reaction is stable for at least two hours.

6. Calculations: Correct background by subtracting the value derived from the 0 glycerol standard from all sample readings. Plot the standard curve (OD 570nm or Fluorescence readings vs. nmol). Apply sample readings to the standard curve. Glycerol concentration can then be calculated:

$$C = Ga / Sv \text{ nmol}/\mu\text{l or } \mu\text{mol}/\text{ml or mM}$$

Where: **Ga** is Glycerol amount from standard curve (nmol).

Sv is the sample volume (before dilution) added in sample wells (μ l).
Glycerol molecular weight: 92.09.



VI. RELATED PRODUCTS:

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| NAD/NADH Quantification Kit | NADP/NADPH Quantification Kit |
| ADP/ATP Ratio Assay Kit | Ascorbic Acid Quantification Kit |
| Glucose Assay Kit | Fatty Acid Assay Kit |
| Ethanol Assay Kit | Uric Acid Assay Kit |
| Pyruvate Assay Kit | Lactate Assay Kit/ II |
| Creatine Assay Kit | Creatinine Assay Kit |
| Ammonia Assay Kit | Free Glycerol Assay Kit |
| Triglyceride Assay Kit | Hemin Assay Kit |
| Choline/Acetylcholine Assay Kit | Total Antioxidant Capacity (TAC) Assay Kit |
| Sarcosine Assay Kit | L-Amino Acid Assay Kit |
| Nitric Oxide Assay Kit | Glutathione Detection Kit s |
| ADP & ATP Colorimetric Kits | ADP & ATP fluorometric Kits |
| Glutamate Assay Kit | Glycerol Assay Kit |
| Cholesterol Assay Kit | HDL & LDL/VLDL Assay Kits |
| Starch Assay Kit | Glycogen Assay Kit |
| NAD/NADH Assay Kit | NADP/NADPH Assay Kit |
| Heme Assay Kit | Ascorbic Acid Assay Kit |
| Calcium Assay Kit | Iron Assay Kit |