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BioVision
BioVision Incorporated

Free Glycerol Colorimetric Assay Kit II

(Catalog # K634-100; 100 assays; Store at -20°C) *rev.12/12*

I. Introduction:

Glycerol is a central component of most lipids. It acts as a backbone for triglycerides and phospholipids, which play an important role in metabolism and cell membrane structure. Due to its low toxicity, glycerol is widely used in the pharmaceutical, food and cosmetic industries. Biovision's Free Glycerol Assay kit II is suitable for measuring free glycerol levels in samples that contain reducing substances, which may interfere with oxidase-based assays. In this assay, Glycerol in the presence of Glycerol Enzyme Mix is converted to an intermediate, which reduces a colorless Probe to a colored product with strong absorbance at 450 nm. Free Glycerol Assay Kit II is simple, rapid and high-throughput adaptable. This assay kit can detect less than 20 μM of free Glycerol in various biological samples.

II. Application:

- Measurement of free glycerol in various tissues/cells.
- Analysis of metabolism and cell signaling.

III. Sample Type:

- Serum, plasma, urine.
- Animal tissues: Liver, muscle, heart etc.
- Cell culture: Adherent or suspension cells.
- Cells & tissue culture supernatant

IV. Kit Contents:

Components	K634-100	Cap Code	Part Number
Glycerol Assay Buffer	25 ml	WM	K634-100-1
Glycerol Enzyme Mix (Lyophilized)	1 vial	Green	K634-100-2
Probe (Lyophilized)	1 vial	Red	K634-100-3
Glycerol Standard (100 mM)	0.2 ml	Yellow	K634-100-4

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom.
- Multi-well spectrophotometer (ELISA reader).

VI. Storage and Handling:

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

VII. Reagent Preparation and Storage Conditions:

- **Glycerol Enzyme Mix**: Reconstitute with 220 µl Glycerol Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze thaw. Keep on ice while in use. Use within 2 months.
- **Probe**: Dissolve with 220 µl dH_2O. Pipette up and down to dissolve completely. Stable for 2 months at -20°C.

VIII. Glycerol Assay Protocol:

1. **Sample Preparation:** Rapidly homogenize tissues (10 mg) or cells (1 x 10^6) with 100 µl ice cold Glycerol Assay Buffer for 10 minutes on ice. Centrifuge at 12000 rpm for 5 min. Collect the supernatant. Add 1-50 µl sample (20-100 µg) per well. Adjust the volume to 50 µl with Glycerol Assay Buffer.
   Notes:
   a. NADH in samples will generate background. For samples having high NADH levels, prepare parallel sample well(s) as background control to subtract background from NADH.
   b. Enzyme in some samples may interfere with the assay. Enzymes may be removed by using 10 kD spin column (BioVision Cat# 1997-25).
   c. For unknown samples, we suggest testing several doses of samples to ensure the readings are within the standard curve range.
2. **Standard Curve Preparation:** Dilute Glycerol Standard to 1 mM (1 nmol/µl) by adding 10 µl of 100 mM Glycerol Standard to 990 µl dH_2O. Mix well. Add 0, 2, 4, 6, 8, 10 µl of 1 mM Glycerol Standard into a series of wells in 96 well plate to generate 0, 2, 4, 6, 8, and 10 nmol/well Glycerol Standard. Adjust volume to 50 µl/well with Glycerol Assay Buffer.
3. **Reaction Mix:** Mix enough reagents for the number of assays (samples and Standards) to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	Background Control Mix
Glycerol Assay Buffer	46 µl	48 µl
Glycerol Enzyme Mix	2 µl	----
Probe	2 µl	2 µl

Add 50 µl of the Reaction Mix to each well containing Standard & test samples. Mix well.

Note: For samples having color or high NADH levels, add 50 µl of Background Control Mix to sample background control well(s). Mix well.

4. **Measurement:** Incubate at 37°C for 60 minutes. Measure OD_{450nm}.
5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the Glycerol Standard Curve. Correct sample background by subtracting the value derived from the background control from sample readings. Apply the corrected sample reading to the Glycerol Standard Curve to get B nmol of Glycerol amount in the sample well(s).

Sample Glycerol concentration = B/V x Dilution Factor = nmol/ml = µM

Where: **B** is the amount of Glycerol in the sample (nmol).
V is the sample volume used in the reaction well (ml).
Glycerol molecular weight: 92.09 g/mole.
Free glycerol in samples can also be expressed in nmol/mg of sample or any other desired method.

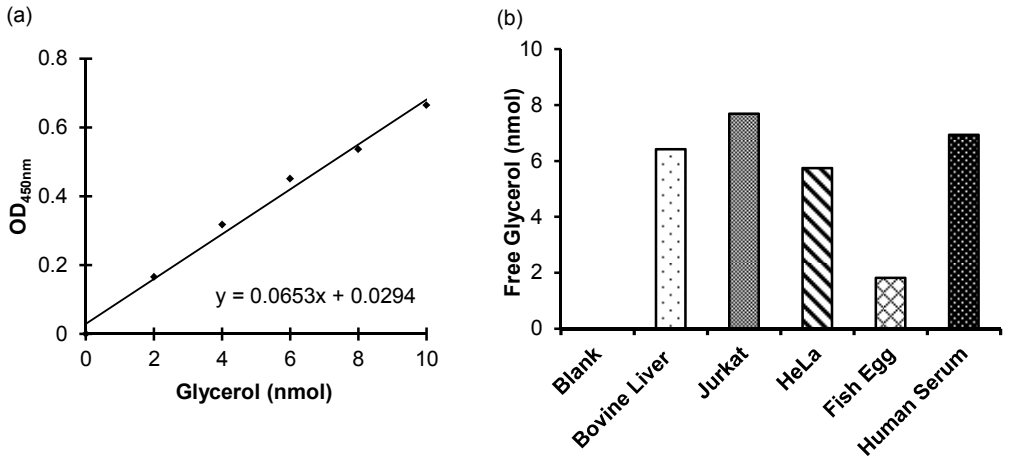

Figure: Glycerol Standard Curve [a]. Measurement of free glycerol in bovine liver (50 µg), Jurkat cells (40 µg), HeLa cells (30 µg), fish egg (58 µg) & human serum (2 µl) [b]. Assays were performed following kit protocol.

IX. RELATED PRODUCTS:

Adipogenesis Assay Kit
Glucose Assay kit
Glucose and Sucrose Assay Kit
Glucose Dehydrogenase Activity Assay Kit
Glucose-6-Phosphate Dehydrogenase Assay Kit
Glucose Uptake Colorimetric Assay
Glucose Uptake Fluorometric Assay
Free Glycerol Assay Kit
Maltose and Glucose Assay Kit
NAD/NADH Quantification Kit
NADP/NADPH Quantification Kit
Triglyceride quantification kit

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