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PicoProbe™ Free Glycerol Fluorometric Assay Kit

rev. 11/15

(Catalog # K643-100; 100 assays; Store at -20°C)

I. Introduction:

Glycerol is a central component for synthesis of all lipids; it acts as a backbone for triglycerides and phospholipids, which plays an important role for cell membrane's structure. Due to its low toxicity, glycerol is widely used in pharmaceutical, food and cosmetic industries. Biovision's PicoProbe™ Free Glycerol Fluorometric Assay kit is simple, sensitive and easy to use. This assay is suitable for measuring trace amount of glycerol in samples, which contain reducing substances that may interfere with oxidase-based assays. In the assay, glycerol reacts with Enzyme Mix to form an intermediate, which is subsequently oxidized with the production of fluorescence. The fluorescence intensity is directly proportional to the amount of glycerol. This assay kit can detect glycerol as low as 1.0 µM.

Glycerol $\xrightarrow{\text{Enzyme Mix}}$ Intermediate $\xrightarrow{\text{Probe}}$ Fluorescence (Ex/Em = 535/587 nm)

II. Application:

- Measurement of glycerol in various tissues/cells
- Analysis of lipid metabolism
- Mechanistic study of cardiovascular diseases

III. Sample Type:

- Animal tissues: e.g., kidney, heart etc.
- Cell culture: Adherent or suspension cells
- Biological fluids: serum, plasma etc.

IV. Kit Contents:

Components	K643-100	Cap Code	Part Number
Glycerol Assay Buffer	25 ml	WM	K643-100-1
PicoProbe™ (in DMSO)	0.4 ml	Blue	K643-100-2
Glycerol Enzyme Mix (Lyophilized)	1 vial	Green	K643-100-3
Glycerol Developer (Lyophilized)	1 vial	Red	K643-100-4
Glycerol Standard (100 mM)	0.2 ml	Yellow	K643-100-5

V. User Supplied Reagents and Equipment:

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

VI. Storage and Handling:

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

VII. Reagent Preparation and Storage Conditions:

- **PicoProbe™:** Ready to use as supplied. Warm to room temperature before use. Store at -20°C.
- **Glycerol Enzyme Mix and Glycerol Developer**: Reconstitute with 220 µl Glycerol Assay Buffer, making sure the material is completely dissolved. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use. Stable for 2 months at -20°C.

VIII. Glycerol Assay Protocol:

1. **Sample Preparation:** Serum samples can be measured directly. Rapidly homogenize tissue (10 mg) or cells (~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. Saliva should be briefly spun down at 5000 rpm for 2 minutes; collect the supernatant for the assay. Add1-50 µl samples into a 96 well plate and bring the volume to 50 µl with Glycerol Assay Buffer.

   Notes:

   A. For unknown samples, we suggest testing several doses of your samples to ensure the readings are within the Standard Curve range.

   B. NADH in samples will generate background. For samples having high NADH levels, prepare parallel sample well(s) as background control.

2. **Standard Curve Preparation:** Dilute 100 mM Glycerol Standard (100 nmol/µl) to 1mM (1000 pmol/µl) by adding 10 µl of 100 mM Glycerol Standard to 990 µl Glycerol Assay Buffer, mix well. Dilute 1 mM Glycerol Standard further to 60 µM (60 pmol/µl) by adding 60 µl of 1mM Glycerol Standard to 940 µl Glycerol Assay Buffer, mix well. Add 0, 2, 4, 6, 8 & 10 µl of the 60 pmol/µl Glycerol Standard into a series of wells in 96-well plate to generate 0, 120, 240, 360, 480, and 600 pmol/well Glycerol Standards. Adjust volume to 50 µl/well with Glycerol Assay Buffer.

   Notes:

   To generate 0, 40, 80, 120, 160, 200 pmol Glycerol standard, dilute 20 µl of 1 mM Glycerol to 980 µl Assay Buffer to generate 20 µM (20 pmol/µL) Glycerol, mix well. Add 0, 2, 4, 6, 8, 10 µl of 20 µM Glycerol, and 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	43 µl	45 µl
PicoProbe™	3 µl	3 µl
Glycerol Enzyme Mix	2 µl	----
Glycerol Developer	2 µl	2 µl

Add 50µl of the Reaction Mix to each well containing the Standards and test samples and 50µl of Background Control Mix to sample background control well(s). Mix well.

4. **Incubation:** Incubate the reaction for 60 min. at room temperature, protected from light.
5. **Measurement:** Measure fluorescence at Ex/Em = 535/587 nm in a microplate reader.
6. **Calculation:** Subtract 0 Standard reading from all readings. Plot the Glycerol Standard Curve. If background control reading is significantly high, subtract the background control reading from sample reading. Apply corrected sample reading to the Glycerol Standard Curve to get B pmol of Glycerol in the sample wells.

Sample Glycerol concentration (C) = B/V x Dilution Factor = pmol/µl = nmol/ml = µM

B = the amount of glycerol in the sample well (pmol).
V = the sample volume used in the reaction well (µl).

Glycerol molecular weight: 92.09 g/mol
Glycerol in sample can also be expressed in pmol/mg or mg/dL of sample.

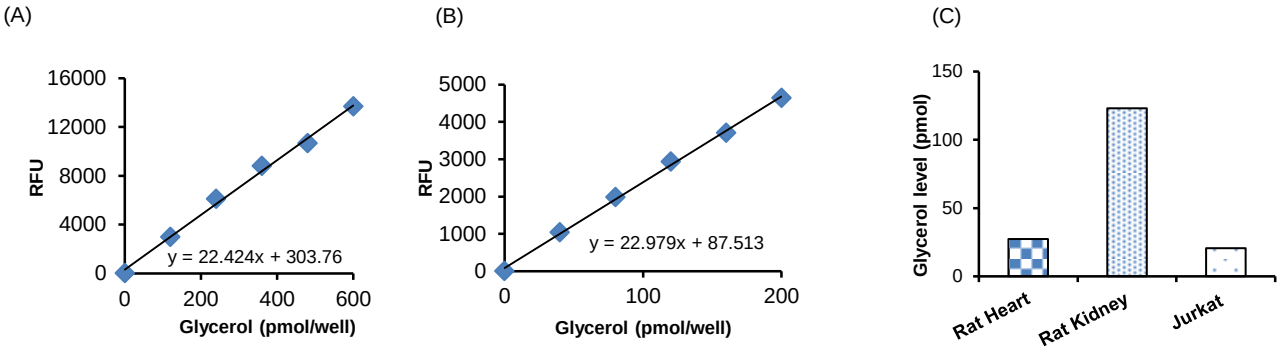

Figure: (A) and (B) Glycerol Standard Curves, (C) measurement of glycerol levels in rat heart (5 µg protein), rat kidney (5 µg) and Jurkat cell lysate (10 µg). Assays were performed according to Kit protocol.

IX. RELATED PRODUCTS:

Free glycerol Assay kit
Free glycerol Colorimetric/Fluorometric Assay kit
Triglyceride Quantification Colorimetric/Fluorometric Assay kit
PicoProbe™ Triglyceride Fluorometric Assay kit
Lipase Activity Colorimetric Assay Kit II
Phosphatidylcholine Colorimetric/Fluorometric Assay kit
NADP/NADPH Quantification Kit
PicoProbe™ NADH Fluorometric Assay kit
Glycerol-3-Phosphate Dehydrogenase Activity Colorimetric Assay Kit
Free Fatty Acid Quantification Colorimetric/Fluorometric Kit
Free Glycerol Colorimetric Assay Kit II
Lipase Activity Colorimetric Assay Kit
Lipase Activity Colorimetric Assay Kit III
NAD/NADH Quantification Kit
Adipogenesis Colorimetric/Fluorometric Assay Kit
Glycerol-3-Phosphate Colorimetric Assay Kit
PicoProbe™ Aldehyde Dehydrogenase Activity Fluorometric Assay Kit
Cholesterol/Cholesteryl Ester Quantification Colorimetric/Fluorometric Kit
HDL and LDL/VLDL Quantification Colorimetric/Fluorometric Kit

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