Product Information

Free Glycerol Fluorometric Assay Kit

I. Kit Contents:

Components	K2094-100 100 assays	Cap Color	Part Number
Glycerol Assay Buffer	25 ml	WM	K2094-C-1
PicoProbeTM (in DMSO)	0.4 ml	Blue	K2094-C-2
Glycerol Enzyme Mix (Lyophilized)	1 Vial	Green	K2094-C-3
Glycerol Developer (Lyophilized)	1 Vial	Red	K2094-C-4
Glycerol Standard (100 mM)	0.2 ml	Yellow	K2094-C-5

II. Introduction:

Glycerol is a central backbone for all lipids such as triglycerides and phospholipids, which is a major component in cell membrane. Because of the low toxicity of glycerol, it is widely used in cosmetic, food and pharmaceutical industries.

The PicoProbe™ Free Glycerol Fluorometric Assay Kit provides a sensitive, easy to use and simple way for detection of trace amount glycerol in various samples based on fluorometric method. In the assay, glycerol reacts with Enzyme Mix to yield an intermediate, which is subsequently oxidized with the production of fluorescence that can be detected at Ex/Em= 535/587 nm. The reducing substances contained in the samples may interfere with oxidase-based assays. The fluorescence intensity is directly proportional to the amount of glycerol. The kit can detect glycerol amount less than 40 pmol.

III. Application:

Measurement of glycerol in various tissues/cells.

Analysis of lipid metabolism.

Mechanistic study of cardiovascular diseases.

IV. Sample Type:

Animal tissues: e.g., kidney, heart etc.

Cell culture: Adherent or suspension cells.

Biological fluids: serum, plasma etc.

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℃, 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:

PicoProbeTM: Ready to use as supplied. Warm to room temperature before use. Store at -20℃.

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℃. Avoid repeated freeze/thaw. Keep on ice while in use. Stable for 2 months at -20℃.

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. Add 1 - 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.

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.

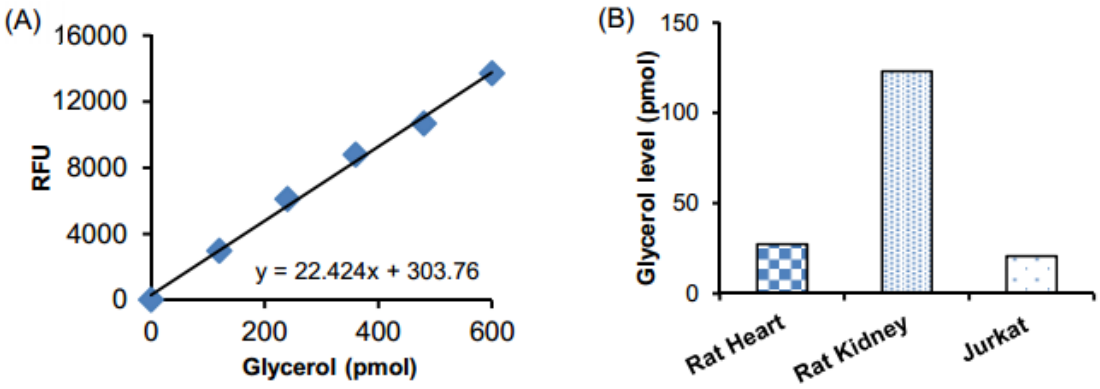

Figure: (A) Glycerol Standard Curve, (B) measurement of glycerol levels in rat heart (5 μg protein), rat kidney (5 μg) and Jurkat cell lysate (10 μg). Assays were performed according to Kit protocol.

Frequently Asked Questions

1. Should medium to be assayed by this kit be stored at -20℃ or 4℃?

We recommend storing the medium at -20℃ or preferably at -80℃ for best results. If the assay will be done in multiple batches, aliquots can be stored to minimize freezing-thawing.

2. What is the exact volume of sample required for this assay?

There is no specific volume we can recommend for the amount any sample to be used since it is completely sample concentration and quality based. It is recommended to do a pilot expt with multiple sample volumes to determine the optimal volume which gives a reading within the linear range of the standard curve. Please refer to the citations for this product to see what other clients have used with similar sample types.

3. What is the shelf life of this kit?

This kit is good for 12 months from the date of shipment in the unopened form when stored at the appropriate temperature and appropriate conditions. After opening and reconstitution, some of the components in this kit are good for 2 months at -20℃. Please refer to the datasheet for storage information and shelf life of each of the components.

4. Why are the standard curve values lower than those shown on the datasheet?

There are multiple factors which influence the signals like the incubation times, room temperature, handling etc. In general, to increase the value of the standards, the incubation time can be increased. As long as the standard curve is linear, it should be fine to use, since all the samples will also be measured under the same conditions on this curve.

5. How are samples normalized against protein concentration?

A protein quantitation assay can be used with the supernatants from cell/tissue lysates or with any other liquid sample in the assay buffer.

6. Is it essential to make a standard curve for every expt, or is one curve/kit enough?

Yes, it is strongly recommended to do the standards every time you do the expt. There is always a chance that something was done differently that day and we do not want any conditions to differ between standards and samples.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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