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Product Information

High Sensitivity Free Glycerol Assay Kit

Catalog Number **MAK270**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Glycerol, also referred to as glycerin or glycerine, is a 3 carbon sugar alcohol that forms the backbone of fatty acids. It is a central component for synthesis of all lipids, and acts as a backbone for triglycerides and phospholipids, which play an important role in cell membrane structure. Due to its low toxicity, glycerol is widely used in pharmaceutical, food, and cosmetic formulations and is the main waste by-product of biodiesel production via transesterification.

The High Sensitivity Free Glycerol Assay Kit is suitable for measuring trace amounts of glycerol in samples, which contain reducing substances that may interfere with oxidase-based assays. The coupled enzyme reaction, results in a fluorometric product (λ_{ex} = 535 nm/ λ_{em} = 587 nm), proportional to the amount of glycerol present. This assay kit is simple and sensitive with the capability to detect <40 pmole of glycerol in a variety of samples.

This kit is suitable for use with serum, plasma, and other biological fluids, and tissue and cell culture samples.

Components

The kit is sufficient for 100 assays in 96 well plates.

Glycerol Assay Buffer 25 mL
Catalog Number MAK270A

High Sensitivity Probe, in DMSO 0.4 mL
Catalog Number MAK270B

Glycerol Enzyme Mix 1 vl
Catalog Number MAK270C

Glycerol Developer 1 vl
Catalog Number MAK270D

Glycerol Standard, (100 mM) 0.2 mL
Catalog Number MAK270E

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate. It is recommended to use white plates with clear bottoms for fluorescence assays.
- Fluorescence multiwell plate reader

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Glycerol Assay Buffer – Allow buffer to come to room temperature before use.

High Sensitivity Probe – Warm to room temperature to thaw the solution prior to use. Store at –20 °C. Upon thawing, the probe is ready-to-use as supplied.

Glycerol Enzyme Mix and Glycerol Developer – Reconstitute with 220 µL of Glycerol Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Keep on ice while in use. Use within 2 months of reconstitution.

Storage/Stability

The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Glycerol Standards for Fluorometric Detection
Dilute 10 µL of the 100 mM Glycerol Standard with 990 µL of Glycerol Assay Buffer to prepare a 1 mM (1,000 pmol/µL) standard solution. Dilute further by adding 60 µL of the 1 mM Glycerol Standard with 940 µL of Glycerol Assay Buffer to prepare a 60 µM (60 pmole/µL) solution. Add 0, 2, 4, 6, 8, and 10 µL of the 60 µM standard solution into a 96 well plate, generating 0 (blank), 120, 240, 360, 480, and 600 pmole/well standards. Add Glycerol Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Fluorometric assays require 50 µL of sample for each reaction (well).

Serum samples can be assayed directly.

Tissue (10 mg) or cells (1×10^6) can be homogenized in 100 µL of ice-cold Glycerol Assay Buffer for 10 minutes on ice. Centrifuge the samples at 12,000 × *g* for 5 minutes to remove insoluble material. Collect the supernatant.

Saliva should be centrifuged at 5,000 × *g* for 2 minutes. Collect the supernatant

Add 1–50 µL of samples into duplicate wells of a 96 well plate. Bring samples to a final volume of 50 µL with Glycerol Assay Buffer.

Notes: For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the range of the standard curve.

NADH present in the sample can generate background. To control background, include a sample control for each sample by omitting the Glycerol Enzyme Mix in the Reaction Mix. The Sample Blank readings can then be subtracted from the sample readings.

Assay Reaction
1. Set up the Master Reaction Mix according to the scheme in Table 1. 50 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	**Samples and Standards**	**Sample Blank**
Glycerol Assay Buffer	43 µL	45 µL
High Sensitivity Probe	3 µL	3 µL
Glycerol Enzyme Mix	2 µL	–
Glycerol Developer	2 µL	2 µL

2. Add 50 µL of the Master Reaction Mix to each sample and standard control well. If using a sample control, add 50 µL of Sample Blank Mix to sample control wells. Mix well using a horizontal shaker or by pipetting.

3. Incubate the plate for 60 minutes at room temperature. Protect the plate from light during the incubation.

4. Measure fluorescence intensity (λ_{ex} = 535/ λ_{em} = 587 nm).

Results

Calculations

The background for the assay is the value obtained for the 0 (blank) Glycerol Standard. Correct for the background by subtracting the blank standard value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Glycerol Standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the Sample Blank value from the sample readings to obtain the corrected fluorescence measurement. Using the corrected fluorescence measurement, determine the amount of Glycerol present in the sample from the standard curve.

Concentration of Glycerol

$$S_a/S_v = C$$

S_a = Amount of Glycerol in the unknown sample (pmole) from standard curve
S_v = Sample volume (μL) added into the wells
C = Concentration of Glycerol in sample

Glycerol molecular weight: 92.09 g/mole

Sample Calculation

Amount of Glycerol (S_a) = 258.4 pmole (from standard curve)
Sample volume (S_v) = 50 μL

Concentration of Glycerol in sample:

$$258.4 \text{ pmole}/50 \ \mu\text{L} = 5.168 \text{ pmole}/\mu\text{L}$$

$$5.168 \text{ pmole}/\mu\text{L} \times 92.09 \text{ pg/pmole} = 475.9 \text{ pg}/\mu\text{L}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use white plates with clear bottoms
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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