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Product Information

Glycerol Assay Kit

Catalog Number **MAK117**
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. Glycerol is widely used in food, beverage, and pharmaceutical formulations and is the main waste by-product of biodiesel production via transesterification.

This kit is suitable for glycerol detection in foods, beverages, pharmaceutical formulations, and biological samples.

In this kit, glycerol concentration is determined by a coupled enzyme assay involving glycerol kinase and glycerol phosphate oxidase, resulting in a colorimetric (570 nm)/fluorometric (λ_{ex} = 535/λ_{em} = 587 nm) product, proportional to the glycerol present. The linear range of detection for this kit is 10–1,000 μM (colorimetric) and 2–50 μM (fluorometric).

Components

The kit is sufficient for 200 assays in 96 well plates.

Assay Buffer Catalog Number MAK117A	24 mL
Dye Reagent Catalog Number MAK117B	0.22 mL
Enzyme Mix Catalog Number MAK117C	0.5 mL
Standard, 100 mM Glycerol Catalog Number MAK117D	0.1 mL
ATP Catalog Number MAK117E	0.25 mL

Reagents and Equipment Required but Not Provided.

- 96-well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is 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.

Storage/Stability

The kit is shipped on dry ice. Storage at –20 °C, protected from light, is recommended. If desired, the Assay Buffer can be stored at 2–8 °C.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of standards and samples. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Procedure

Bring all reagents to room temperature prior to use except for the Enzyme Mix. The Enzyme Mix should be kept on ice while in use.

The Assay Buffer may contain a precipitate after storage at –20 °C. This precipitate does not impact the performance of the reagent. Equilibrating the solution at room temperature for several hours or warming in a water bath from 37–60 °C should dissolve most of the precipitate. Allow the buffer to return to room temperature before beginning the assay.

It is recommended to run samples and standards in duplicate.

Glycerol Standards for Colorimetric Detection
Dilute Standards as indicated in Table 1. Diluted samples can be stored at 2–8 °C and kept for future use.

Table 1.
Colorimetric Standards

Number	Glycerol Standard (100 mM)	Water	Final Glycerol Concentration
1	10 µL	990 µL	1.0 mM
2	6 µL	994 µL	0.6 mM
3	3 µL	997 µL	0.3 mM
4	0 µL	1,000 µL	0 mM

Glycerol Standards for Fluorometric Detection
Mix 10 µL of the 100 mM Standard with 990 µL of water to create a 1 mM working standard. Dilute the 1 mM working standard as indicated in Table 2.

Table 2.
Fluorometric Standards

Number	Glycerol Standard (1 mM)	Water	Final Glycerol Concentration
1	50 µL	950 µL	0.050 mM
2	30 µL	970 µL	0.030 mM
3	15 µL	985 µL	0.015 mM
4	0 µL	1000 µL	0 mM

Assay Reaction

1. Transfer 10 µL of the appropriate standards and 10 µL of samples into separate wells of a 96 well plate.

2. Set up the Master Reaction Mix according to the scheme in Table 3. 100 µL of the Master Reaction Mix is required for assay well. The Master Reaction Mix should be prepared fresh each time the reaction is run.

Table 3.
Master Reaction Mix

Reagent	Volume
Assay Buffer	100 µL
Enzyme Mix	2 µL
ATP	1 µL
Dye Reagent	1 µL

3. Add 100 µL of the Master Reaction Mix to each of the blank, standard, and sample wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 20 minutes at room temperature. Protect the plate from light during the incubation.

4. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (FLU, λ_{ex} = 535/λ_{em} = 587 nm).
   Notes: If the sample absorbance is higher than the absorbance of standard number 1 (1 mM for the colorimetric assay), dilute the sample in water and repeat the assay.

   Similarly, if the sample fluorescence intensity is higher than the fluorescence intensity of standard number 1 (0.05 mM for the fluorimetric assay), dilute the sample in water and repeat the assay.

   In either case, multiply the results by the dilution factor.

Results

Calculations

The background for either assay is the value obtained for the 0 glycerol standard (blank). Correct for the background by subtracting the blank value from all readings. Use the values obtained from the appropriate glycerol standards to plot a standard curve and determine the slope using linear regression fitting.

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

Concentration of Glycerol

The concentration of glycerol present in the samples may be determined from the standard curve using the equations below.

$$C = \frac{(A_{570})_{sample}}{Slope}$$

$$C = \frac{(FLU)_{sample}}{Slope}$$

Slope = Slope determined from standard curve
C = Concentration of glycerol in sample (mM)

1 mM Glycerol is equal to 9.2 mg/dL or 92 ppm

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 black plates with clear bottoms. For colorimetric assays, use clear plates
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 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 master 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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