Product Information

Free Glycerol Colorimetric/Fluorometric Assay Kit

I. Kit Contents:

Components	K2134-100 100 assays	Cap Color	Part Number
Glycerol Assay Buffer	25 ml	WM	K2134-C-1
Glycerol Probe (in DMSO, Anhydrous)	0.2 ml	Red	K2134-C-2
Glycerol Enzyme Mix (lyophilized)	1 vial	Green	K2134-C-3
Glycerol Standard (100 mM)	0.2 ml	Yellow	B7776

II. Introduction:

Glycerol is a polyol and acts as a backbone to all lipids known as triglycerides. Glycerol is widely used in solvents, beverages, foods, pharmaceutical and cosmetic products, etc. Quantification of glycerol causes broad interest for research and development.

The Free Glycerol Colorimetric/Fluorometric Assay Kit provides a sensitive, fast and convenient way for detection of free glycerol in various samples based on colorimetric and fluorometric method. In the assay, glycerol is enzymatically oxidized to produce a product which reacts with the probe to yield fluorescence (Ex/Em = 535/587 nm) and color (λ = 570 nm). The kit can detect 50 pmol-10 nmol (or 1 ~ 10000 µM range) glycerol in various samples.

III. Storage and Handling:

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

IV. Reagents Preparation and Storage Conditions:

Glycerol Enzyme Mix: Dissolve in 220 µl Assay Buffer. Aliquot and store at –20℃. Use within two months.

Glycerol Probe: Briefly warm at 37℃ for 1 - 2 min to dissolve. Mix well. Store at –20℃. Use within two months.

V. Glycerol Assay Protocol:

1. Standard Curve Preparation:

For the colorimetric assay, add 10 µl of the glycerol standard to 990 µl of Assay Buffer to generate 1 mM glycerol standard, mix well. Add 0, 2, 4, 6, 8, 10 µl into each well individually. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of glycerol Standard.

For the fluorometric assay, dilute the Glycerol Standard to 0.01 - 0.1 mM with the Assay Buffer (Detection sensitivity is 10 - 100 fold higher for a fluorometric than a colorimetric assay). Follow the protocol as for the colorimetric assay.

2. Sample Preparation: Treat serum sample with Carrez Clarification Reagent Kit to remove anti-oxidants before running the assay. Add 10 µl of supernatant in a 96-well plate. Adjust the volume to 50 µl with Assay Buffer. Cells (10^6 cells) or tissue samples (10 mg) can be homogenized in 500 µl Assay Buffer. Centrifuge sample at 10,000 x g for 10 min. Collect supernatant. Add 1 - 50 µl of the extracted sample in a 96-well plate. Adjust the volume to 50 µl with Assay Buffer. Certain cell or tissue samples may need to be treated with Carrez Clarification Reagent Kit. We suggest testing several dilutions of your sample to make sure the readings are within the standard curve range.

3. Reaction Mix: Mix enough reagent for the number of samples and standards to be performed: For each well, prepare a total 50 µl Reaction Mix:

Assay Buffer	46 µl
Glycerol Probe	2 µl

Glycerol Enzyme Mix 2 µl

The fluorometric assay is ~10 times more sensitive than the colorimetric assay. Use 0.4 µl of the probe per reaction to decrease background/increase detection sensitivity significantly.

4. Add 50 µl of the Reaction Mix to each well containing standard and samples. Mix well. Incubate at room temperature for 30 min, protect from light.

5. Measure $OD_{570\ nm}$ for the colorimetric assay or Ex/Em = 535/590 nm for the fluorometric assay in a microtiter plate reader. The reaction is stable for at least two hrs.

6. Calculations: Correct background by subtracting the value derived from the 0 glycerol standard from all sample readings. Plot the standard curve (OD 570 nm or Fluorescence readings vs. nmol). Apply sample readings to the standard curve. Glycerol concentration can then be calculated:

C = Ga / Sv nmol/µl or mol/ml or mM

Where: Ga is Glycerol amount from standard curve (nmol).

Sv is the sample volume (before dilution) added in sample wells (µl).

Glycerol molecular weight: 92.09.

General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in d atasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not	• Use of partially thawed components	• Thaw and resuspend all components before preparing the

follow a linear pattern for Standard curve	• Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

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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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