# Free Glycerol Colorimetric/Fluorometric Assay Kit (BN00854)

(Catalog #BN00854; 100 assays; Store at -20° C)

## I. Introduction:

Glycerol is widely used in foods, beverages, solvents, pharmaceutical and cosmetic products, etc. There is broad interest in quantification of glycerol for research and development. Assay Genie's Glycerol Assay Kit provides a sensitive, easy assay to measure free glycerol concentration in various samples. In the assay, glycerol is enzymatically oxidized to generate a product which reacts with the probe to generate color (λ = 570 nm) and fluorescence (Ex/Em = 535/587 nm). The kit can detect 50 pmol-10 nmol (or 1~10000 µM range) of glycerol in various samples.

## II. Kit Contents:

| Components | BN00854 | Cap Code | Part Number |
|---|---|---|---|
| Glycerol Assay Buffer | 25 ml | WM | BN00854-1 |
| Glycerol Probe (in DMSO, Anhydrous) | 0.2 ml | Red | BN00854-2A |
| Glycerol Enzyme Mix (lyophilized) | 1 vial | Green | BN00854-4 |
| Glycerol Standard (100 mM) | 0.2 ml | Yellow | BN00854-5 |

## III. Storage and Handling:

Store kit at –20° C, 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° C. Use within two months.

**Glycerol Probe**: Briefly warm at 37° C for 1-2 min to dissolve. Mix well. Store at –20° C. 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 (Cat. # BN01025) 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 (Cat. # BN01025). 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:

46 µl Assay Buffer
2 µl Glycerol Probe*
2 µl Glycerol Enzyme Mix

* 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 µmol/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.

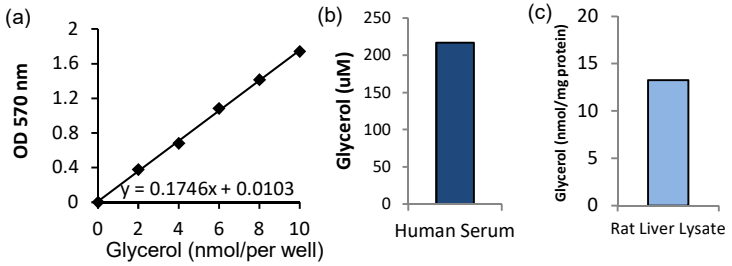


Figure: (a) Glycerol Standard Curve. (b) Measurement of Free glycerol in pooled human serum (10 µl). Sample was treated with Carrez Clarification Reagent Kit (Cat. # BN01025) before analysis. (c) Measurement of Free glycerol in rat liver lysate (500 µg). Assay was performed following the kit protocol.

***FOR RESEARCH USE ONLY! Not to be used on humans.***

## GENERAL TROUBLESHOOTING GUIDE:

| Problems | Cause | Solution |
|---|---|---|
| Assay not working | • Use of ice-cold assay buffer | • Assay buffer must be at room temperature |
| | • Omission of a step in the protocol | • Refer and follow the data sheet precisely |
| | • Plate read at incorrect wavelength | • Check the wavelength in the data sheet and the filter settings of the instrument |
| | • Use of a different 96-well plate | • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates |
| Samples with erratic readings | • Use of an incompatible sample type | • Refer data sheet for details about incompatible samples |
| | • Samples prepared in a different buffer | • Use the assay buffer provided in the kit or refer data sheet for instructions |
| | • Samples were not deproteinized (if indicated in datasheet) | • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated |
| | • Cell/ tissue samples were not completely homogenized | • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope |
| | • Samples used after multiple free-thaw cycles | • Aliquot and freeze samples if needed to use multiple times |
| | • Presence of interfering substance in the sample | • Troubleshoot if needed, deproteinize samples |
| | • Use of old or inappropriately stored samples | • Use fresh samples or store at correct temperatures till 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 | • Always check the expiry date and store the components appropriately |
| | • Allowing the reagents to sit for extended times on ice | • Always thaw and prepare fresh reaction mix before use |
| | • Incorrect incubation times or temperatures | • Refer datasheet & verify correct incubation times and temperatures |
| | • Incorrect volumes used | • Use calibrated pipettes and aliquot correctly |
| Readings do not follow a linear pattern for Standard curve | • Use of partially thawed components | • Thaw and resuspend all components before preparing the reaction mix |
| | • Pipetting errors in the standard | • 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 tubes |
| | • Standard stock is at an incorrect concentration | • Always refer the dilutions in the data sheet |
| | • Calculation errors | • Recheck calculations after referring the data sheet |
| | • Substituting reagents from older kits/ lots | • Use fresh components from the same kit |
| Unanticipated results | • Measured at incorrect wavelength | • Check the equipment and the filter setting |
| | • Samples contain interfering substances | • Troubleshoot if it interferes with the kit |
| | • Use of incompatible sample type | • Refer data sheet to check if sample is compatible with the kit or optimization is needed |
| | • Sample readings above/below the linear range | • 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. | | |