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Product Information

00254 *Fluoroselect*™ Glycerol Kit

Product description
Glycerol (glycerin or glycerine, C3H5(OH)3) is widely used in foods, beverages and pharmaceutical formulations. It is also a main by-product of biodiesel production. Simple, direct and automation ready procedures for measuring glycerol concentrations find wide applications.
Sigma-Aldrich's glycerol assay uses a single working reagent that combines glycerol kinase, glycerol phosphate oxidase and color reactions in one step. The color intensity of the reaction product at 570 nm, or fluorescence intensity at $\lambda_{ex/em}$ = 530/590 nm is directly proportional to glycerol concentration in the sample.

Detection ranges and limits
Linear detection range: 2 -100 µM, 18-920 µg/dL or 0.18-9.20 ppm.
Sensitive and accurate. Use as little as 10 µL samples.
Simple and convenient. The procedure involves addition of a single working reagent and incubation for 20 min at room temperature.
Improved reagent stability. The optimized formulation has greatly enhanced the reagent and signal stability.

Equipment required but not included
Z805491-1EA *FluoroSELECT*™ Single channel fluorometer λ_{ex} 530 nm; λ_{em} 590 nm
Z805823-100EA Glass vials for *FluoroSELECT*™ fluorometer

Components
1. 24 mL Assay Buffer
2. 500 µL Enzyme Mix
3. 250 µL ATP
4. 220 µL Dye Reagent
5. 100 µL Glycerol Standard

The kit is sufficient for approximately 200 assays.

Storage conditions
Store at -20°C

Procedure
1. Standard. Prepare 1 mM standard by mixing 5 µL of the provided standard with 495 µL distilled H_2O. Then mix 50 µL of the 1mM standard with 450 µL H_2O to obtain 100 µM glycerol standard. In separate mini-glass tubes add 10 µL H_2O ("Blank"), 10 µL 100 µM glycerol standard ("Std"), and 10 µL sample.
2. Prepare enough working reagent for all assay tubes, by mixing per tube: 100 µL assay buffer, 2 µL enzyme mix, 1 µL ATP and 1 µL dye reagent in a clean Eppendorf tube. Then add 100 µL working reagent to each tube and mix. Incubate for 20 min in the dark.
3. Switch on the reader. To calibrate the reader, place the "Blank" tube into the sample holder. Press "Calibrate", "Assay 1", then "Blank". Reader starts measuring. Press left arrow on "<-Std -> ", until the window shows "100.000". Place the "Std" tube into the sample holder. Press "Measure". The reader shows "Calibration Finished". Press "Return".
4. Measure. Place the sample tube into the sample holder. Press "Measure", "Assay 1", "Measure". The glycerol concentration (µM) will be displayed in the window. Record the data, or press "Save" to save the data for later retrieval. Press "Return" and then press "Measure" for the next sample.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.