Tribioscience

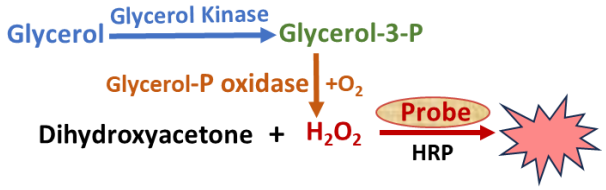
Glycerol Fluorometric Assay Kit (TBS2204F, 100 assays; store at -20°C)

DESCRIPTION

Glycerol is a central component for the synthesis of all lipids and acts as a backbone for triglycerides and phospholipids. It is released from triglycerides as the product of lipolysis. The measurements of circulating glycerol are useful parameters to evaluate lipolysis under various conditions in clinical studies.

Tribioscience's Glycerol Fluorometric Assay is a robust, simple, and high throughput method based on coupled enzyme reactions. Glycerol is phosphorylated and oxidized to generate hydrogen peroxide, which is catalyzed and reacts with the probe measured by the fluorometric method at Ex/Em = 530/590 nm.

ASSAY PRINCIPLE



APPLICATIONS

This assay can detect as low as 0.2 µg/mL of glycerol in a variety of biological samples, including food, beverage, cell culture medium, cell lysate, tissues and serum, and plasma samples.

KIT CONTENTS

Name	Size (100 tests)
Glycerol standard (25 µg/mL)	250 µL
Enzyme Mix (10X)	800 µL
Glycerol assay buffer	12 mL
Glycerol Probe	80 µL

STORAGE AND HANDLING

Store kit at -20°C. Shelf life of 12 months.

ASSAY PROTOCOL

Except Enzymes, warm all the components to room temperature before use. Briefly centrifuge all small vials prior to opening.

1. Sample Preparation:
Serum and plasma can be tested directly. Tissue and cells can be homogenized in glycerol assay buffer. Centrifuge for 2 to 5 minutes at top speed of a microcentrifuge. Collect the supernatant.

2. Standard Curve Preparations:

Tube	Glycerol Addition (µL)	Assay Buffer (µL)	Glycerol Conc (µg/mL)
1	100 µL of stock	100 µL	12.5
2	100 µL of Tube#1	100 µL	6.25
3	100 µL of Tube#2	100 µL	3.12
4	100 µL of Tube#3	100 µL	1.56
5	100 µL of Tube#4	100 µL	0.78
6	100 µL of Tube#5	100 µL	0.39
7	100 µL of Tube#6	100 µL	0.19
8	0	100 µL	0

Add 20 µL/well of the standards and the samples. Use black plate for fluorometric assay.

3. Glycerol Reaction Mix: Prepare enough mix for 100 tests as the table below and mix well.

Glycerol Reaction Mix	Volume
Glycerol assay buffer	7.2 mL
Enzyme Mix (10X)	800 µL
Glycerol Probe	60 µL

Add 80 µL of the Glycerol Reaction Mix to each well containing the glycerol standards and the test samples. Tap plate lightly to mix.

4. Incubation: Incubate at 37°C for 30 minutes with gentle agitation and protect from light.

5. Measurement: Measure at Ex/Em = 530/590 nm.

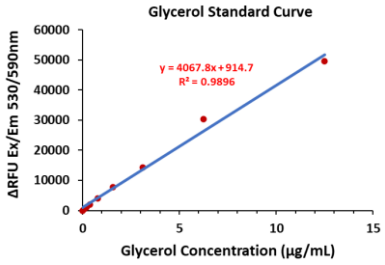
6. Calculation: Correct background by subtracting the value of the 0 Glycerol standard (blank) from all standard readings. Plot the value against standard concentration. Determine the slope using linear regression fitting.

Typical standard curve is y=Ax + B, A is the slope and B is the y-intercept. X is concentration.

Glycerol = N* [(RFU_{sample}-RFU_{blank})-B] /A (µg/mL)

RFU_{sample} and RFU_{blank} are fluorescence values of the sample and buffer. **(Note:** If unknown sample results over standard curve range, dilute sample with assay buffer. Repeat the assay; multiply the results by the dilution factor ***N***.)

The typical glycerol standard curve:



RELATED PRODUCTS

Glycerol Colorimetric Assay (TBS2204C)
Triglyceride Colorimetric Assay (TBS2205C)
Triglyceride Fluorometric Assay (TBS2205F)
Pyruvate Colorimetric Assay (TBS2023C)
Pyruvate Fluorometric Assay (TBS2023F)
LDH Cytotoxicity Assay (TBS2002)
ATP Colorimetric/Fluorometric Assay (TBS2010)
ADP Colorimetric/Fluorometric Assay (TBS2020)
Cell Count Kit -8 (TBS2022)
XTT Cell Viability Assay (TBS2021)
Caspase-3 Colorimetric Assay (TBS2030)
Thiol Fluorometric Assay (TBS2026)
GSH Assay (TBS2028)
Homocysteine Fluorometric Assay (TBS2091)
NNMT Inhibitor Screening Assay (TBS2097)
NNMT Activity Fluorometric Assay (TBS2098)
G6PDH Activity Colorimetric Assay (TBS2102)
Cytochrome c Reductase Activity Assay (TBS2116)

RESEARCH USE ONLY

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