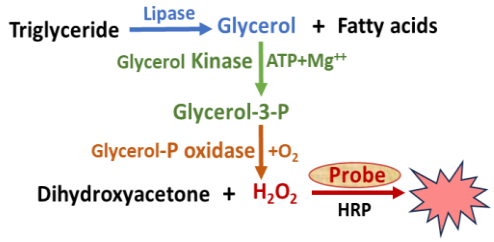
Triglyceride Colorimetric Assay Kit (TBS2205C, 100 assays; store at -20°C)

DESCRIPTION

Triglycerides (TG) are esters of glycerol and three fatty acids. They are bound to proteins and transported as macromolecular particles called lipoproteins. Triglyceride concentrations are useful in the diagnosis of diabetes mellitus, pancreatitis, heart disease, and other diseases. High triglyceride levels increase the risk for heart disease, strokes, and type-2 diabetes.

Tribioscience's Triglyceride Colorimetric Assay kit is designed to be a robust, simple, and high throughput method based on coupled enzymatic reactions. Triglyceride is hydrolyzed to produce glycerol and fatty acids. The glycerol is oxidized to generate hydrogen peroxide which reacts with the probe measured by colorimetric method at OD = 570 nm.

ASSAY PRINCIPLE



APPLICATIONS

Direct Assays: As low as 1 mg/dL of Triglyceride in serum, cell culture, tissues and other biological samples.

KIT CONTENTS

Name	Size (100 tests)
Triglyceride standard (200 mg/dL)	150 µL
Enzyme1 Mix	480 µL
Enzyme2	12 µL
Triglyceride assay buffer	12 mL
Triglyceride Probe	80 µL
Lipoprotein lipase	110 µL

STORAGE AND HANDLING

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

PRECAUSTIONS:

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

ASSAY PROTOCOL

Briefly place the triglyceride standard tube in a hot water bath (80-100°C) and then vortex to make it completely dissolved in solution.

1. Sample Preparation:

Serum and plasma can be tested directly. Tissue and cells can be homogenized in 5% NP-40 solution in water. Heat to 80-100°C in a water bath for 2-5 minutes, cool down to room temperature. Repeat the heating one more time to solubilize all triglyceride. Centrifuge for 2 minutes at top speed of a microcentrifuge to remove all insoluble materials. Dilute the supernatant with assay buffer and add 10 µL/well of the samples.

2. Standard Curve Preparations:

Tubes	Triglyceride Addition (µL)	Assay Buffer (µL)	Triglyceride Concentration (mg/dL)
1	50 µL Stock	50 µL	100
2	50 µL of Tube#1	50 µL	50
3	50 µL of Tube#2	50 µL	25
4	50 µL of Tube#3	50 µL	12.5
5	50 µL of Tube#4	50 µL	6.25
6	50 µL of Tube#5	50 µL	3.12
7	50 µL of Tube#6	50 µL	1.56
8	0	50 µL	0

Add 10 µL/well of the standards, or sample, or blank control. Use clear plate for colorimetric assay.

3. Addition of Lipoprotein lipase

For 100 assays, dilute 100 µL of Lipoprotein lipase with 900 µL of assay buffer, add 10 µL to the wells. For sample glycerol background control, do not add lipase to the wells, add 10 µL/well of assay buffer instead. Incubate at 37°C for 30 minutes with gentle agitation.

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

Triglyceride Reaction Mix	Volume
Triglyceride assay buffer	7.470 mL
Enzyme1 Mix	460 µL
Enzyme2	10 µL
Triglyceride Probe	60 µL

Add 80 µL of the Reaction Mix to each well containing the Triglyceride Standard and test samples and sample glycerol background control. Tap plate lightly to mix.

4. Incubation: Incubate at 37°C for 1-10 minutes with gentle shaking and protect from light.

5. Measurement: Measure OD at 570 nm for colorimetric assay.

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

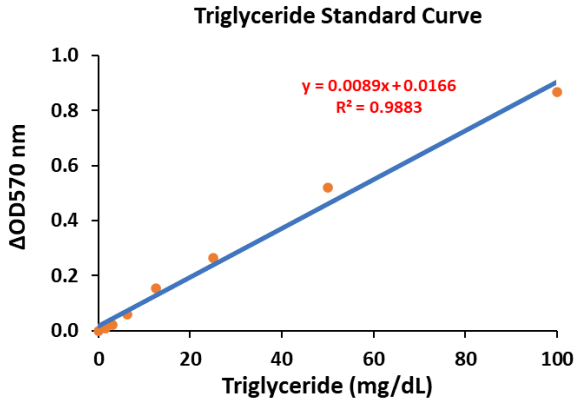
Typical standard curve is y=Ax+B, A is the slope; B is the y-intercept; X is concentration.

Triglyceride =N* [(OD_{sample}-OD_{blank}) -B]/A (mg/dL)

OD_{SAMPLE} and OD_{blank} are optical density values of the sample and buffer. N is dilution factor. (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.)

If the sample glycerol background control is significant, subtract it from sample readings.

Typical standard curve of triglyceride:



RELATED PRODUCTS

Triglyceride Fluorometric Assay (TBS2205F)
Glycerol Colorimetric Assay (TBS2204C)
Glycerol Fluorometric Assay (TBS2204F)
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)

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