Triglyceride Assay Kit (Colorimetric)

LS-K302-200 (200 Tests) • **Store at -20°C**

LSBio
LifeSpan BioSciences, Inc.

Introduction

TRIGLYCERIDE, also known as TRIACYLTRIGLYCERIDE or TRIACYLGLYCERIDE, is the main constituent in vegetable oil and animal fats. Triglycerides play an important role as energy sources and transporters of dietary fat. In the human body, high levels of triglycerides in the bloodstream have been linked to atherosclerosis, heart disease and pancreatitis. Simple, direct and automation-ready procedures for measuring triglyceride concentrations find wide applications in research and drug discovery. LSBio's triglyceride assay uses a single Working Reagent that combines triglyceride hydrolysis and glycerol determination in one step, in which a dye reagent is oxidized to form a colored product. The color intensity at 570nm is directly proportional to triglyceride concentration in the sample.

Key Features

- Sensitive and accurate. Use as little as 10 µL samples. Linear detection range 0.01 mmol/L to 1.0 mmol/L (0.88 mg/dL to 88.5 mg/dL) triglyceride.
- Simple and convenient. The procedure involves addition of a single working reagent and incubation for 30 min at room temperature, compatible for HTS assays. Improved reagent stability. The optimized formulation has greatly enhanced the reagent and signal stability.

Applications

- Direct Assays: triglyceride in biological samples (e.g. serum and plasma).
- Drug Discovery/Pharmacology: effects of drugs on triglyceride metabolism.

Components

Component	K302-200
	200 Tests
Assay Buffer	24 mL
ATP	250 µL
Dye Reagent	220 µL
Enzyme Mix	500 µL
Lipase	1000 µL
Standard (equivalent to 100 mmol/L Triglyceride)	100 µL

Materials Not Supplied

Pipetting devices, centrifuge tubes, clear flat bottom 96-well plates (e.g. Corning Costar) and plate reader.

Storage

The kit is shipped on ice. Store all kit components at -20 °C.

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Assay Procedure

Note: SH-group containing reagents (e.g. mercaptoethanol, DTT) may interfere with this assay and should be avoided in sample preparation.

1. Equilibrate all components to room temperature. Keep thawed Lipase and Enzyme Mix in a refrigerator or on ice. Dilute Standard in distilled water as follows. Transfer 10 µL diluted standards into wells of a clear 96-well plate. Diluted standards can be used for future assays when stored refrigerated.

No	STD + H_2O	Vol (µL)	Triglyceride (mmol/L)
1	10µL + 990µL	1000	1.0
2	6µL + 994µL	1000	0.6
3	3µL + 997µL	1000	0.3
4	0µL + 1000µL	1000	0

Serum and plasma samples should be diluted 5-fold in dH2O and are assayed directly. Cells and other solid samples can be solubilized in 5% Triton X-100. Transfer 10 µL samples into separate wells of the 96-well plate.

2. Prepare Working Reagent for each well, by mixing 100 µL AssayBuffer, 2 µL Enzyme Mix, 5 µL Lipase, 1 µL ATP and 1 µL Dye Reagent in a clean tube. Transfer 100 µL Working Reagent into standards and sample wells. Tap plate to mix.

3. Incubate 30 min at room temperature. Read optical density at 570nm (550-585nm).

Note: 1. if the Sample OD is higher than the Standard OD at 1.0 mmole/L triglyceride, dilute sample in water and repeat the assay. Multiply by the dilution factor n.

Calculations

Subtract OD_{H2O} (water, #4) from the standard OD values and plot the OD against standard concentrations. Determine the slope using linear regression fitting. The triglyceride concentration of Sample is calculated as

$$[\text{Triglyceride}] = \frac{OD_{SAMPLE} - OD_{H2O}}{\text{Slope}} \times n \quad (\text{mmol/L})$$

OD_{SAMPLE} and OD_{H2O} are optical density values of the sample and the water blank (# 4). n is the dilution factor. For example serum or plasma samples are diluted 5-fold prior to assay, n = 5. Conversions: 1 mmol/L triglyceride equals 88.5 mg/dL or 10 ppm.

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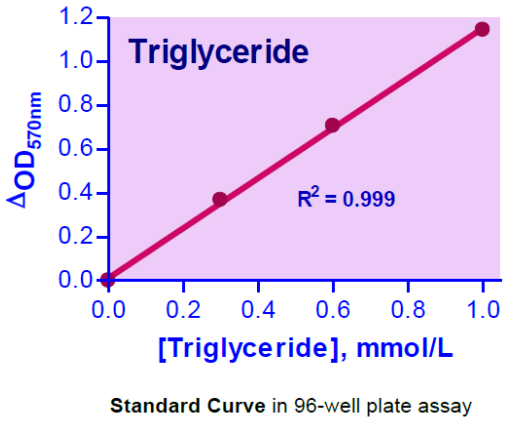
Sample Data



Standard Curve in 96-well plate assay

Version: V.08.09.2018