# EnzyChrom™ Triglyceride Assay Kit (Cat# ETGA-200)

## Quantitative Colorimetric Triglyceride Determination at 570nm

## DESCRIPTION

*TRIGLYCERIDE,* also known as TRIACYLTRIGLYCERIDE or TRIACYL-GLYCERIDE, 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. BioAssay Systems' 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.

## KIT CONTENTS

**Assay Buffer:** 24 mL **ATP**: 250 µL **Dye Reagent**: 220 µL
**Enzyme Mix:** 500 µL **Lipase**: 1000 µL
**Standard**: 100 µL (equivalent to 100 mmol/L Triglyceride)

**Storage conditions**. The kit is shipped on ice. Store all components at -20°C. Shelf life of 12 months after receipt.

**Precautions**: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

## PROCEDURES

*Note*: (1) SH-group containing reagents (e.g. mercaptoethanol, DTT) may interfere with this assay and should be avoided in sample preparation; (2) if sample contains glycerol, use BioAssay Systems' EnzyChrom™ Glycerol Assay Kit (Cat# EGLY-200) to determine glycerol concentration and subtract the glycerol value to yield triglyceride concentration.

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 $dH_2O$ 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 Assay Buffer, 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*.

## CALCULATION

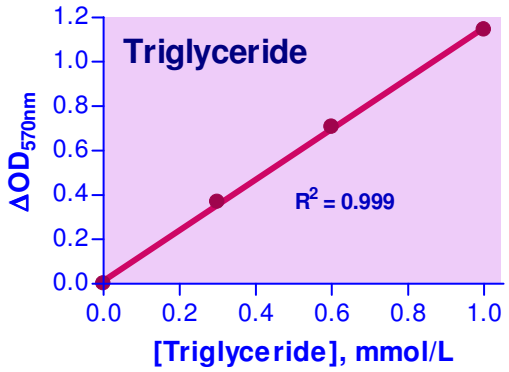
Subtract $OD_{H2O}$ (water, #4) from the standard OD values and plot the OD against standard concentrations. Determine the slope using linear regression fitting. The triglyceride concentration of Sample is calculated as

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

$OD_{SAMPLE}$ 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.

## MATERIALS REQUIRED, BUT NOT PROVIDED

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




**Standard Curve** in 96-well plate assay

## PUBLICATIONS

1. Kim HS et al (2010). Hepatic-specific disruption of SIRT6 in mice results in fatty liver formation due to enhanced glycolysis and triglyceride synthesis. Cell Metab. 12(3):224-36
2. Tam J et al (2010). Peripheral CB1 cannabinoid receptor blockade improves cardiometabolic risk in mouse models of obesity. J Clin Invest. 120(8):2953-66
3. Guo H et al (2011). Anthocyanin inhibits high glucose-induced hepatic mtGPAT1 activation and prevents fatty acid synthesis through PKCζ. J Lipid Res. 52(5):908-22.