Triglyceride Assay Kit

Triglyceride Assay Kit can be used to measure Triglyceride in serum, plasma and other biological samples.

Catalog number: ARG81628

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INTRODUCTION

A triglyceride (TG, triacylglycerol, TAG, or triacylglyceride) is an ester derived from glycerol and three fatty acids (from tri- and glyceride). Triglycerides are the main constituents of body fat in humans and other vertebrates, as well as vegetable fat. They are also present in the blood to enable the bidirectional transference of adipose fat and blood glucose from the liver, and are a major component of human skin oils.

There are many different types of triglyceride, with the main division between saturated and unsaturated types. Saturated fats are "saturated" with hydrogen—all available places where hydrogen atoms could be bonded to carbon atoms are occupied. These have a higher melting point and are more likely to be solid at room temperature. Unsaturated fats have double bonds between some of the carbon atoms, reducing the number of places where hydrogen atoms can bond to carbon atoms. These have a lower melting point and are more likely to be liquid at room temperature.

In the human body, high levels of triglycerides in the bloodstream have been linked to atherosclerosis, heart disease and stroke. However, the relative negative impact of raised levels of triglycerides compared to that of LDL:HDL ratios is as yet unknown. The risk can be partly accounted for by a strong inverse relationship between triglyceride level and HDL-cholesterol level. But the risk is also due to high triglyceride levels increasing the quantity of small, dense LDL particles. [Wikipedia: Triglyceride]

PRINCIPLE OF THE ASSAY

This Triglyceride Assay Kit provides a simple, and rapid procedure for measuring Triglyceride concentration in samples. This 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. The concentration of Triglyceride in the sample is then determined by comparing the signals of samples to the standard.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
Assay Buffer	24 ml (Ready to use)	-20°C
Triglyceride Standard (100 mM)	100 µl	-20°C
Enzyme Mixture	500 µl (Ready to use)	-20°C
Lipase	1 ml (Ready to use)	-20°C
ATP	250 µl (Ready to use)	-20°C
Dye Reagent	220 µl (Ready to use)	-20°C

The kit is shipped on ice. Store all components at -20°C in dark upon received. Shelf life of around 12 months after receipt.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 570 nm.
- Flat bottomed 96-well microplate.
- Pipettes and pipette tips
- Deionized or distilled water.

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- The kit is shipped on ice. Store all components at -20°C in dark. Shelf life of around 12 months after receipt.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Keep thawed Lipase and Enzyme Mixture in a refrigerator or on ice before use. All other materials should be equilibrated to room temperature (RT) before use.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Please Notice the following guideline:

(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, the endogenously glycerol concentration has to be determined and subtract the glycerol value to yield triglyceride concentration.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at -20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Cell/Tissue lysate- Lysis sample by 5% Triton X-100 buffer. Lysis samples by homogenization or by sonication on ice. Then centrifuge at 14,000 rpm for 10 min at 4°C. Use clear supernatant for assay. Collect Samples and assay immediately or aliquot & store samples at -80°C. Avoid repeated freeze-thaw cycles.

Note:

- Serum and plasma samples should be diluted 5X with distilled water and are assayed directly.

- If the initial assay found samples contain Triglyceride higher than1 mM, the samples can be diluted with distilled water and then re-assay the samples. For the calculation of the concentrations this dilution factor (n) has to be taken into account. The sample must be well mixed with the diluents buffer before assay. (It is recommended to do pre-test to determine the suitable dilution factor).

REAGENT PREPARATION

- Standard:

  - Dilute 100 mM Triglyceride Standard solutions with distilled water to 1 mM, 0.6 mM and 0.3 mM as following table, and use distilled water serves as zero standard (0 µM).

Standard No.	Standard Conc. mM (mmol/L)	Distilled water (µl)	100 mM Standard (µl)
S1	1	990	10
S2	0.6	994	6
S3	0.3	997	3
S0	0	1000	0

Diluted standards can be used for future assays when stored refrigerated.

- Working Reagent:

  For each reaction combine the following (*Prepare before use)*:

  100 µL of Assay Buffer

  2 µL of Enzyme Mixture

  5 µL of Lipase

  1 µL of ATP

  1 µL Dye Reagent.

  Mix well, transfer 100 µl of Working Reagent to each sample and standard wells.

ASSAY PROCEDURE

Keep thawed Lipase and Enzyme Mixture in a refrigerator or on ice before use. All other materials should be equilibrated to room temperature (RT) before use, each vial should be mixed thoroughly without foaming and briefly centrifuge tubes prior to use. Each sample requires a sample blank.

1. Add **10 µl** of each (diluted) **sample** and **standard** into appropriate well in a flat bottomed 96 well plate.
2. Add **100 µl** of the **Working Reagent** to each well.
3. Gently tap the plate to ensure thorough mixing. Incubate for 30 min at room temperature in dark.
4. Read the OD with a microplate reader at 570 nm (550 - 585nm) immediately.

Summary:

	Assayed sample	Standard	Blank (S0)
Sample	10 µl	-	-
Standard	-	10 µl	-
dH2O	5 µl	-	10 µl
Working Reagent	100 µl	100 µl	100 µl
Mix well and incubate for 30 min at RT in dark.			
Read the OD with a microplate reader at 570nm immediately.			

CALCULATION OF RESULTS

1. Subtract OD dH2O (water, S0) from the standard OD values and plot the OD against standard concentrations. Determine the slope using linear regression fitting.
2. The sample Triglyceride concentration is calculated as follows:

   [Triglyceride] (mM) =

   N X [(OD $_{Sample}$ – OD $_{Blank}$) / Slope

   Note:

   OD $_{Sample}$: OD value of Assayed sample well

   OD $_{Blank}$: OD value of Blank well

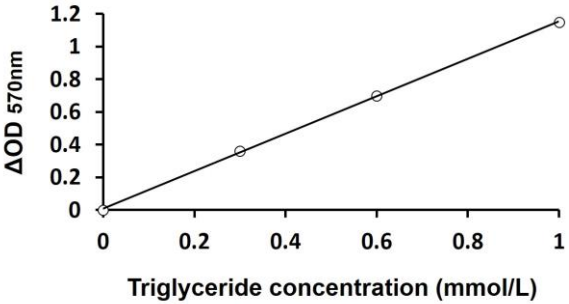
   Slope: Calculated from standard ODs using linear regression fitting

   N = dilution factor (if sample has been diluted before assay)

   For example serum or plasma samples are diluted 5-fold prior to assay, n = 5.
3. If the calculated Triglyceride concentration is >1 mM dilute sample in distilled water and repeat assay. Multiply result by the dilution factor N.
4. Conversions: 1 mM Triglyceride equals 88.5 mg/dL, or 10 ppm.

EXAMPLE OF ASSAY

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

Linear detection range:

0.01 mM to 1.0 mM (0.88 mg/dL to 88.5 mg/dL)

0.02

The minimum detectable dose (MDD) of Triglyceride was:

0.01 mM (0.88 mg/dL)