MANUAL

RayBio® Triglyceride Colorimetric Assay Kit

Catalog #: MA-TG

ISO 13485:2016

Introduction

Triglycerides are a family of lipids absorbed from the diet and produced endogenously from carbohydrates. Triglycerides constitute 95% of fat stocked in tissues and their main role is to provide energy to cells. The synthesis of triglycerides occurs in two main locations: the intestine, where dietary fats are processed, and the liver, where carbohydrates are converted into triglycerides. Subsequently, these triglycerides are transported in the bloodstream by chylomicrons and very low-density lipoproteins (VLDL). Elevated levels of triglycerides have been associated with high risk of severe atherosclerosis. High triglyceride levels and hyperlipidemia, in general, can be attributed to genetic factors or can arise as a consequence of various disorders, such as diabetes mellitus, nephrosis, biliary obstruction, and metabolic disorders associated with endocrine imbalances.

RayBio® Triglyceride Colorimetric Assay Kit provides a simple, reproducible, and sensitive tool of measuring triglyceride concentration in plasma, serum, cell lysates and other biological liquid samples. This assay employs a coupled enzymatic reaction system that combines multiple steps. Initially, lipase catalyzes the hydrolysis of triglycerides into free fatty acids and glycerol. The released glycerol is then phosphorylated by adenosine triphosphate (ATP) with glycerol kinase (GK) to produce glycerol-3-phosphate and adenosine diphosphate. Glycerol-3-phosphate is subsequently oxidized by dihydroxyacetone phosphate (DAP) via glycerolphosphate oxidase producing hydrogen peroxide (H_2O_2) which then reacts with the Colorimetric Probe to form a pink colored product. The optical density measured at 510nm is directly proportional to the concentration of triglyceride present in the sample.

Storage

The entire kit may be stored at 2–8 °C for up to 6 months from the date of shipment. For prepared reagent storage, see table below.

Component	Size / Description	Storage After Preparation
Microplate (Item A)	A 96-well (12 strips x 8 wells) plate	RT*
Sample Buffer	10 ml	2–8 °C
Triglyceride Standard	1 vial (100 µl of 200 mg/dL)	2–8 °C
Enzyme Mix Solution	20 ml	2–8 °C

RT = room temperature

**Return unused wells to the pouch containing desiccant pack, reseal along entire edge.*

Additional Materials Required

1. Microplate reader capable of measuring absorbance at 510 nm
2. Precision pipettes to deliver 2 µl to 1 ml volumes
3. Distilled or deionized water
4. Tubes to prepare sample dilutions
5. Incubator at 37°C

Sample Tips and General Considerations

NOTE: Optimal methods of sample preparation will need to be determined by each researcher empirically based on researched literature and knowledge of the samples.

- If not using fresh samples, freeze samples as soon as possible after collection.
- Avoid multiple freeze-thaw cycles. If possible, sub-aliquot samples prior to initial storage.
- It is strongly recommended to add a protease inhibitor cocktail to cell and tissue lysate samples.
- Avoid sonication of 1 ml or less as this can quickly heat and denature proteins.
- Most samples will not need to be concentrated. If concentration is required, a spin column concentrator with a chilled centrifuge is recommended.

1. Cell lysates can be prepared as follows:

For attached cells, remove supernatant from cell culture, wash cells twice with cold 1X PBS (for suspension cells, pellet the cells by spinning down the cells at 1,000 x g for 10 min) making sure to remove any remaining PBS before adding lysis buffer. Solubilize the cells at $2x10^7$ cells/ml in lysis buffer containing protease inhibitors. Pipette up and down to resuspend cells and rock the lysates gently at 2–8 °C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 14,000 x g for 10 minutes.
It is recommended that sample protein concentrations should be determined using a total protein assay. Lysates should be used immediately or aliquot and stored at -70 °C. Thawed lysates should be kept on ice prior to use.
General tips for preparing lysate samples can be viewed on the online Resources page of the website: https://www.raybiotech.com/tips-on-sample-preparation/

2. Plasma samples:

Collect blood with an anticoagulant such as citrate, EDTA or oxalate and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer. Sample should be tested immediately or frozen at -80°C for storage. Typically, normal human plasma has triglyceride concentrations in the range of 40-160 mg/dL (male) or 35-135 mg/dL (female).

3. Serum samples:

Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C for storage. Typically, normal human serum has triglyceride concentrations in the range of 40-160 mg/dL (male) or 35-135 mg/dL (female).

Note:
The levels of triglyceride vary between different samples. Optimal dilution factors for each sample must be determined by the investigator. Use Sample Buffer to dilute samples.

Standard Preparation

To prepare a dilution series of standard in the concentration range of 0 mg/dL – 200 mg/dL (see Table below),

1. Label 8 microtubes #1 through 8 which will contain the following concentrations: 200, 100, 50, 25, 12.5, 6.25, 3.125, 0 mg/dL.
2. Pipette 80 µL Triglyceride Standard into tube #1, this is the 200 mg/dL standard.
3. Pipette 40 µL deionized water into labeled tube #2 – tube #8.
4. To make the 100 mg/dL standard, pipette 40 µL of the tube #1 into the tube labeled #2. Mix thoroughly.
5. Repeat this step with each successive concentration, preparing a dilution series as shown in the Table below. Each time, use 40 µl of the prior concentration until the 3.125 mg/dL in tube #7 is reached. Mix each tube thoroughly before the next transfer. Standards should be prepared fresh, mixed thoroughly and used immediately.

Labeled Tubes	Triglyceride Standard (µL)	Deionized Water (µL)	Standard Conc. (mg/dL)
1	80 µL	0 µL	200
2	40 µL of Tube #1	40 µL	100
3	40 µL of Tube #2	40 µL	50
4	40 µL of Tube #3	40 µL	25
5	40 µL of Tube #4	40 µL	12.5
6	40 µL of Tube #5	40 µL	6.25
7	40 µL of Tube #6	40 µL	3.125
8	0 µL	40 µL	0

Assay Procedure

Each triglyceride standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add 10 µL of the diluted Triglyceride Standards or Diluted Samples to the 96-well microtiter plate.
2. Initiate the reaction by adding 200 µL of Enzyme Mix Solution to each well.
3. Cover with the plate cover. Carefully shake the plate for a few seconds to mix.
4. Incubate the plate for 10 minutes at 37°C.
5. Measure the absorbance at 510nm using a plate reader.

Calculation of Results

Subtract the blanks

Average the absorbance value of the blank wells (Standard 0mg/dL) and subtract this from the absorbance values of all the other wells. These are the corrected absorbance.

Plotting the standard curves

Make a plot of corrected absorbance at 510nm as a function of triglyceride concentration.

Determination of sample triglyceride concentration

Triglyceride (mg/dL) = $\frac{OD_{Sample} - OD_{Blank}}{Slope} \times DF$

OD_{Sample} = Optical density (OD) reading of the Sample

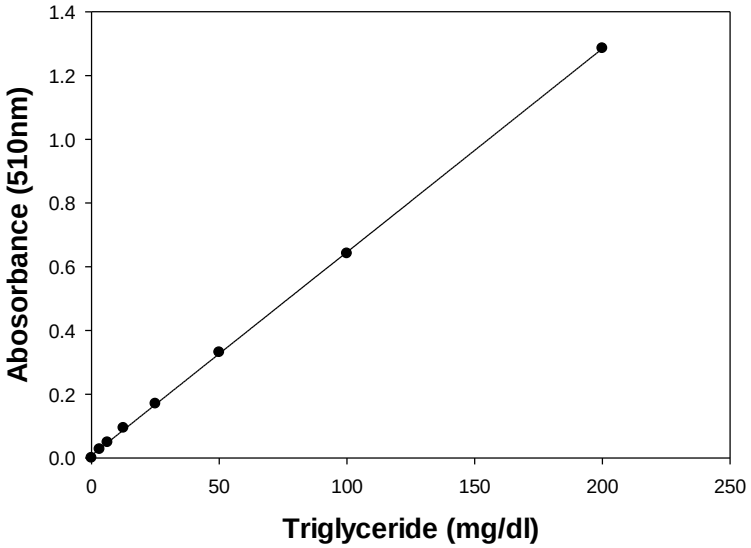
OD_{Blank} = Optical density (OD) reading of the Blank (Standard 0mg/dL)

Slope is from the plot of triglyceride concentration vs. Absorbance shown in Typical data below

DF = Sample Dilution factor (DF = 1 for undiluted Samples)

Note: If the calculated triglyceride concentration of the Sample is higher than 200mg/dL, dilute the Sample in Sample Buffer and repeat the assay.

A. Typical Data



These standard curves are for demonstration only. A standard curve must be run with each assay.

B. Reproducibility

Intra-assay Precision (Precision within an assay):
To assess intra-assay precision, 16 wells per sample (total of 4 samples) were tested on a single plate. The intra-assay coefficient of variation was found to be 1.9%.

Inter-assay Precision (Precision between assays):
To assess inter-assay precision, 4 samples were tested in separate assays (n=4). The inter-assay coefficient of variation was found to be 3.6%.

This product is for research use only.