



Triglyceride Assay (TG)

Cat. No. 8498
100 Tests in 96-well plate

Introduction

A triglyceride is an ester derived from glycerol and three fatty acids. Triglycerides, as major components of very low density lipoprotein and chylomicrons, play an important role as transporters of fatty acids and energy sources. The measurement of triglyceride levels is considered to be useful in the diagnosis of diabetes mellitus, pancreatitis, heart disease and other medical diseases. This colorimetric assay is based on enzymatic hydrolysis of triglycerides, in which the formed glycerol is measured by coupled enzyme reaction. The color intensity of the reaction product at 540nm is directly proportional to the triglyceride level in the sample.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8498a	1	Assay buffer	10 mL	4°C
8498b	1	Triglyceride standard	0.1 mL	4°C
8498c	1	Cofactor mix	0.2 mL	-20°C
8498d	1	Developer	1.6 mL	-20°C
8498e	1	Lipase	0.2 mL	-20°C
8498f	1	Enzyme mix	0.3 mL	-20°C

Product Use

The Triglyceride Assay kit measures the triglyceride level of different types of samples, such as serum, plasma and lysate. This product is for research purposes only and not for use in animals, humans, or diagnostic procedures.

Quality Control

Data from the Triglyceride Assay kit measuring triglyceride solutions with concentrations ranging from 6.25 to 200 mg/dL show a linear relationship between OD_{540nm} and triglyceride concentration (Figure 1).

Shipping

Shipped on dry ice.

Procedure (96-well plate)

A. Preparation of triglyceride standard

1. Obtain 7 test tubes and label them #1 through #7, add 25 μL of assay buffer (8498a) into #2-7 tube.
2. Add 25 μL of the triglyceride standard (8498b) into tube #1 and tube #2, mix well in tube #2 to get the 100 mg/dL triglyceride standard.
3. Transfer 25 μL of the 100 mg/dL triglyceride standard from tube #2 to tube #3 and mix well to get the 50 mg/dL triglyceride standard.
4. Repeat step 3 for tubes #4-6 to serially dilute the triglyceride standards. Do not add any triglyceride to tube #7, which serves as the blank.
5. Obtain a 96-well test plate, prepare 2 replicates (A, B) of each triglyceride standard by aliquoting 10 μL /well of each triglyceride standard into duplicate wells of the 96-well test plate, according to the following plate format:

	#1	#2	#3	#4	#5	#6	#7
A	200mg/dl	100mg/dl	50mg/dl	25mg/dl	12.5mg/dl	6.25mg/dl	Blank
B	200mg/dl	100mg/dl	50mg/dl	25mg/dl	12.5mg/dl	6.25mg/dl	Blank

B. Preparation of test samples

1. Serum and plasma can be directly used for measuring triglyceride level.
2. Cell or tissues can be homogenized in 4 volumes of the PBS containing 1% Triton X-100. Centrifuge the samples at $10,000 \times g$ for 10 minutes at 4°C to remove insoluble material. The soluble fraction may be assayed directly.
3. Samples should be serially diluted to make sure the readings are within the standard curve range. Prepare test samples to a final volume of 10 μL /well on the 96-well flat bottom plate.

C. Working reagent preparation and measurements

1. For each well of reaction, prepare working reagent by mixing 68 μL assay buffer (8498a), 2 μL cofactor mix (8498c), 16 μL developer (8498d), 1 μL LPL (8498e) and 3 μL enzyme mix (8498f).
2. Add 90 μL of working reagent mix into each well of the 96-well plate containing triglyceride standard, samples and blank.
3. Incubate for 20 minutes at room temperature in dark. Read the absorbance at 540 nm with an ELISA plate reader.

D. Calculations

1. Subtract the $\text{OD}_{540\text{nm}}$ value of the blank from the $\text{OD}_{540\text{nm}}$ values obtained with all other standard and samples to get $\Delta\text{OD}_{540\text{nm}}$ value.
2. Based on the calibrated $\Delta\text{OD}_{540\text{nm}}$ of the triglyceride standard, make a standard curve by plotting $\Delta\text{OD}_{540\text{nm}}$ as a function of triglyceride concentration (See Figure 1 for a typical standard curve). Determine the equation and R^2 value of the trend line.

3. If you suppose the equation of the trend line of the standard curve is $y = Ax + B$, you can calculate the triglyceride concentration of test samples as follows:

$$[\text{Triglyceride}] = \frac{\Delta\text{OD}_{540\text{nm}} - B}{A}$$

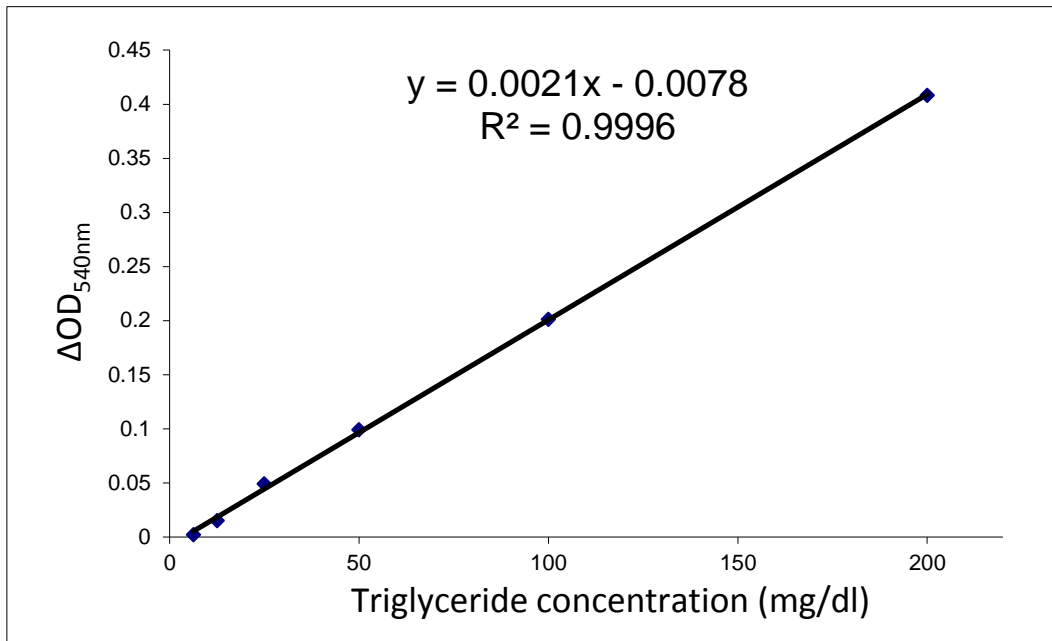


Figure1. A typical triglyceride standard curve measured by the ScienCell™ Triglyceride Assay kit