Product Information

Triglyceride Quantification Colorimetric/Fluorometric Kit

I. Kit Contents:

Components	K2127-100 100 assays	Cap Color	Part Number
Triglyceride Assay Buffer	25 ml	WM	K2127-C-1
Triglyceride Probe (in DMSO, anhydrous)	0.2 ml	Red	K2127-C-2
Lipase	1 Vial	Blue	K2127-C-3
Triglyceride Enzyme Mix (lyophilized)	1 Vial	Green	K2127-C-4
Triglyceride Standard (1 mM)	300 µl	Yellow	K2127-C-5

II. Introduction:

Triglycerides (TG) is an ester derived from three fatty acids and one glycerol and are the main constituent of LDL, VLDL, vegetable oil and animal fat. TG plays an important role in transporting fatty acids and acts as an energy source. TG are broken down into fatty acids and glycerol, both of which can serve as substrates for metabolic pathways and energy producing. High blood levels of TG are associated with pancreatitis, atherosclerosis, heart disease and stroke.

The Triglyceride Quantification Colorimetric/Fluorometric Kit provides a sensitive, fast and convenient way for detection of TG levels in various biological samples based on colorimetric and fluorometric method. In the assay, TG are converted to free fatty acids and glycerol, which is then oxidized to generate a product which reacts with Triglyceride Probe to yield fluorescence (Ex/Em = 535/587 nm) and color (spectrophotometry at λ = 570 nm). The assay can detect 2 pmol - 10nmol (or 2 - 10000 µM range) of triglyceride in various biological samples. The kit can also detect monoglycerides and diglycerides.

III. User Supplied Reagents and Equipment:

96-well white plate (fluorometric) or clear plate (colorimetric).

Multi-well spectrophotometer (Absorbance/Fluorescence reader).

IV. Storage and Handling:

Store kit at -20℃, protect from light. Warm Triglyceride Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening.

V. Reagent Preparation and Storage Conditions:

Triglyceride Standard: Frozen storage may cause the Triglyceride Standard to separate from the aqueous phase. To re-dissolve, keep the cap tightly closed, place in a hot water bath (~ 80 - 100℃) for 1 min or until the standard looks cloudy, vortex for 30 sec., the standard should become clear. Repeat the heat and vortex one more time. The Triglyceride Standard is now completely in solution, and ready to use.

Triglyceride Probe: Ready to use as supplied. Warm by placing in a 37℃ bath for 1 - 5 min to thaw the DMSO solution before use. (Note: DMSO tends to be a solid after -20℃ storage, even when left at room temperature- so need to melt for a few min. at 37℃). Store at -20℃, protect from light. Use within two months.

Triglyceride Enzyme Mix: Dissolve in 220 µl Triglyceride Assay Buffer. Aliquot and store at -20℃. Use within two months.

Lipase: Dissolve in 220 µl Triglyceride Assay Buffer. Aliquot and store at -20℃. Use within two months.

VI. Triglyceride Assay Protocol:

1. Sample Preparation: Add 2 - 50 µl test samples to a 96-well plate. Adjust the volume to 50 µl/well with Triglyceride Assay Buffer. We suggest using different volumes of sample to ensure readings are within the Standard Curve range. A background control should be performed by replacing 2 µl Lipase with 2 µl Triglyceride Assay Buffer (see section 3). The background should be subtracted from all readings. Endogenous compounds in the sample may interfere with the assay, so it is suggested to spike samples with a known amount of Standard (4 nmol) to ensure accurate determinations of Triglyceride in your sample.

Note: Serum contains 0.1 - 6 mM triglyceride, which can be tested directly. For tissue (~ 100 mg), cells (~ 10 million) or other nonaqueous samples, homogenize in 1 ml solution containing 5 % NP-40 in water, slowly heat the samples to 80-100 ℃ in a water bath for 2-5 min. or until the NP-40 becomes cloudy, then cool down to room temperature. Repeat the heating one more time to solubilize all triglyceride. Centrifuge for 2 min. (top speed using a microcentrifuge) to remove any insoluble material. Dilute 10 fold with dH_2O before the assay.

2. Standard Curve Preparation: For the colorimetric assay, Dilute 40 µl of the 1 mM Triglyceride into 160 µl Triglyceride Assay Buffer, mix to generate 0.2 mM Triglyceride Standard. Add 0, 10, 20, 30, 40, 50 µl of the 0.2 mM Triglyceride Standard into a series of wells. Adjust volume to 50 µl/well with Triglyceride Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of Triglyceride Standard.

For the fluorometric assay, dilute the Triglyceride Standard to 0.02 mM with the Triglyceride Assay Buffer (Detection sensitivity is 10 - 100 fold higher for a fluorometric than a colorimetric assay). Follow the procedure as the colorimetric assay.

3. Lipase: Add 2 µl of Lipase to each Standard and sample well. Mix and incubate 20 min. at room temperature to convert triglyceride to glycerol and fatty acid.

Note: If samples contain glycerol, do a sample background control, omit the Lipase to determine glycerol background only, not triglyceride.

4. Triglyceride Reaction Mix: Mix enough reagent for the number of assays to be performed: For each well, prepare a total 50 µl Reaction Mix:

Triglyceride Assay Buffer	46 µl
Triglyceride Probe	2 µl
Triglyceride Enzyme Mix	2 µl

Add 50 µl of the Reaction Mix to each well containing the Triglyceride Standard, samples and background control(s). Mix well. Incubate at room temperature for 30 - 60 min. (60 min. gives slightly better result) protect from light.

Note: For the fluorometric assay, use 0.4 µl/well of the Probe to decrease the background readings, therefore increase detection sensitivity.

5. Measurement: Measure absorbance at 570 nm for colorimetric assay or fluorescence at Ex/Em = 535/590 nm for fluorometric assay in a microtiter plate reader. The reaction is stable for at least two hr.

6. Calculations: Subtract 0 Standard reading from all readings. If sample background control reading is significant then subtract the sample background control reading from sample reading. Plot the TG Standard Curve. For unspiked samples, apply the corrected OD to the TG Standard Curve to get B nmol of TG in the sample well.

Sample TG concentration (C) = B/V X D nmol/µl or mM

Where: B is the amount of TG from Standard Curve (nmol).

V is the sample volume added into the reaction well (µl).

D is the sample dilution factor.

Note: For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading.

For spiked samples, TG amount in sample well (B) =$(OD_{\text{sample (corrected)}}/ (OD_{\text{sample + TG Std(corrected)}} - OD_{\text{sample (corrected)}}))$* TG Spike (pmol).

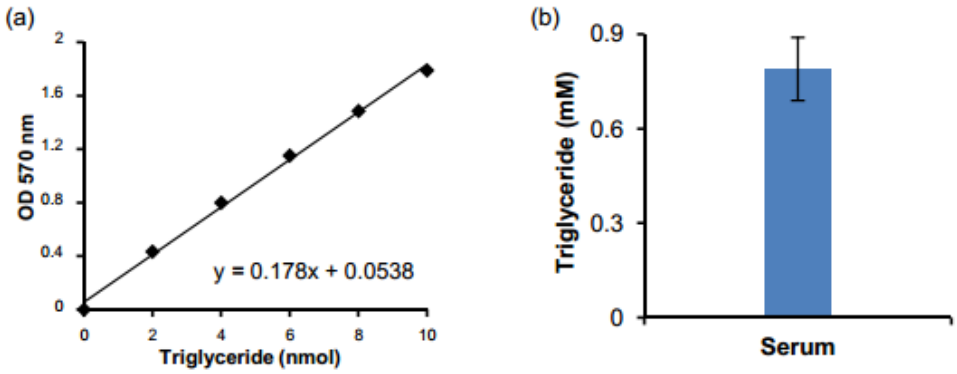

Figure: (a) Triglyceride Standard Curve. (b) Determination of Triglyceride in pooled normal human serum. Serum sample (3 µl) was spiked with a known amount of Triglyceride as internal Standard (4 nmol) and assayed according to the kit protocol. Calculated concentration of triglyceride: 0.79 ± 0.1 mM; (70 ± 8.8 mg/dl).

Frequently Asked Questions:

1. How specific this kit is for measuring triglycerides?

The lipase generates glycerol as well as other products from phospholipids. Glycerol kinase phosphorelates glycerol which is further reduced to develop color. Glucokinase specificity is very high as is the further enzymatic processing, which will not react with phospholipids. When we used phosphatidyl choline, for example, we did not get any color showing that the kit is very specific.

2. What can be done if the lysed cells are not dissolving?

The amount of 5 % NP-40 in water used can be increased. Also, the temperature can be reaised above 80℃ to get the particles into solution in addition to repeating the heating and cooling for 2 cycles.

3. What could be the explanation for negative OD values but positive Bodipy staining in cells?

The kit can detect 2 pmol - 10 nmol (or 2 - 10000 uM range) of triglyceride in various samples. It could be that the Bodipy staining is showing total lipids in these cells but the amount of triglycerides is low. The fluorometric version of this assay using the same kit is 10x more sensitive than the colorimetric one and could be chosen to see if readings make sense. Also, it is crucial to check the instrument settings while measuring the samples. Use the correct filter for 570 nm detection.

4. How much Triglyceride is there is serum samples?

Typically serum levels of Triglyceride can range between 0.1 - 6 mM, individual experimental findings may vary.

5. How do we convert mM to mg/ml?

A solution strength of 1 mol/L is represented as 1mM, so for example, 50 mM is 50 mmol/L (millimoles per liter). Divide by the molecular weight of the substance (average triglyceride molecular weight in your sample) to convert from moles to grams. Then mM/mol weight can be converted to get mg/L. For this assay, since it is difficult to say the molar mass of triglyceride, unless which specific one is present in the sample is known, the accepted most common way of reporting the data is as mM or µmol/ml.

6. Is it generally possible to maintain the samples in NP-40/water at -20℃ and measure their triglyceride concentration again? Additionally, are the triglycerides concentrated on the surface layer of the supernatant or distributed in the whole supernatant after the boiling step?

It is possible that triglycerides aggregate upon freezing and thawing while in an aqueous solution and stick to the walls of the tube which can skew the results. There could be a layer of fat/oil after boiling. Once the sample is centrifuged, the liquid can be collected in a fresh tube and thoroughly vortexed so that when the sample is added to a well, a homogenous solution is pipetted.

7. Can lesser than 10 million cells be used for this assay?

Less cells can be used, but the yield of triglycerides might be less. The number of cells depends on the amount of triglycerides in them. If less cells are used the volume of NP40-water can be scaled down proportionately.

8. What is the shelf life of this kit?

This kit is good for 12 months from the date of shipment in the unopened form when stored at the appropriate temperature and appropriate conditions. After opening and reconstitution, some of the components in this kit are good for 2 months at -20℃. Please refer to the datasheet for storage information and shelf life of each of the components.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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