Triglyceride Quantification Colorimetric/Fluorometric Kit (BN00846)

(Catalog # BN00846; 100 assays; Store at -20°C)

I. Introduction:

Triglycerides (TG) are the main constituent of vegetable oil, animal fat, LDL and VLDL, and play an important role as transporters of fatty acids as well as serving as an energy source. TG are broken down into fatty acids and glycerol, after which both can serve as substrates for energy producing and metabolic pathways. High blood levels of TG are implicated in atherosclerosis, heart disease and stroke as well as in pancreatitis. The Triglyceride Quantification Kit provides a sensitive, easy assay to measure TG concentration in a variety of samples. In the assay, TG are converted to free fatty acids and glycerol. The glycerol is then oxidized to generate a product which reacts with the probe to generate color (spectrophotometry at λ = 570 nm) and fluorescence (Ex/Em = 535/587 nm). The kit can detect 2 pmol-10nmol (or 2-10000µM range) of triglyceride in various samples. The kit also detects monoglycerides and diglycerides.

II. Application:

- Measurement of triglycerides in various tissues, cells & other biological fluids
- Mechanistic study of cardiovascular diseases
- Analysis of lipid metabolism

III. Sample Type:

- Animal tissues: Liver, pancreas, heart etc.
- Cell culture: adipocytes etc.
- Biological fluids: serum, plasma etc..

IV. Kit Contents:

Components	BN00846	Cap Code	Part Number
Triglyceride Assay Buffer	25 ml	WM	BN00846-1
Triglyceride Probe (in DMSO, anhydrous)	200 µl	Red	BN00846-2A
Lipase	1 vial	Blue	BN00846-4
Triglyceride Enzyme Mix (lyophilized)	1 vial	Green	BN00846-5
Triglyceride Standard (1 mM)	0.3 ml	Yellow	BN00846-6

V. User Supplied Reagents and Equipment:

- 96-well white plate (fluorometric) or clear plate (colorimetric)
- Multi-well spectrophotometer (Absorbance/Fluorescence reader)

VI. Storage and Handling:

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

VII. 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°C) 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°C bath for 1-5 min to thaw the DMSO solution before use. (**Note:** DMSO tends to be a solid after -20°C storage, even when left at room temperature- so need to melt for a few min. at 37°C). Store at –20°C, protect from light. Use within two months.
- **Triglyceride Enzyme Mix:** Dissolve in 220 µl Triglyceride Assay Buffer. Aliquot and store at -20 °C. Use within two months.
- **Lipase:** Dissolve in 220 µl Triglyceride Assay Buffer. Aliquot and store at -20 °C. Use within two months.

VIII. 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 non-aqueous samples, homogenize in 1 ml solution containing 5 % NP-40 in water, slowly heat the samples to 80-100 °C 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:

	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) = $\left(\frac{(\mathrm{OD}_{\mathrm{sample\ (corrected)}})}{(\mathrm{OD}_{\mathrm{sample\ +\ TG\ Std(corrected)}}) - (\mathrm{OD}_{\mathrm{sample(corrected)}})}\right) * TG\ Spike\ (\mathrm{nmol})$

Triglyceride molecular weight: 885.4 g/mol

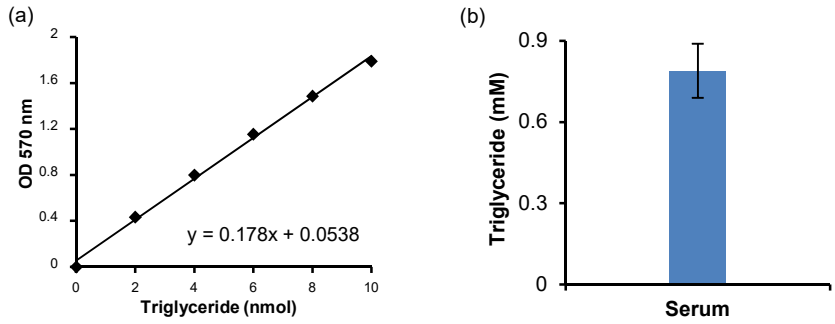

Figure: (a) Triglyceride Standard Curve. (b) Determination of Triglyceride in pooled normal human serum. Serum sample (3 µl) was spiked with a known amount of Triglyceride as internal Standard (4 nmol) and assayed according to the kit protocol. Calculated concentration of triglyceride: 0.79 ± 0.1 mM;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

FOR RESEARCH USE ONLY! Not to be used on humans.