

Triglyceride Quantification Kit

(Catalog #K622-100; 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 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 colorimetric (spectrophotometry at $\lambda = 570 \text{ nm}$) and fluorometric (Ex/Em = 535/587 nm) methods. The kit can detect 2 pmol-10 nmol (or 2~10000 μM range) of triglyceride in various samples.

II. Kit Contents:

Components	K622-100	Cap Code	Part Number
Triglyceride Assay Buffer	25 ml	WM	K622-100-1
Triglyceride Probe (lyophilized)	1 vial	Red	K622-100-2
Dimethylsulfoxide (DMSO, Anhydrous)	0.4 ml	Brown	K622-100-3
Lipase	1 vial	Blue	K622-100-4
Triglyceride Enzyme Mix (lyophilized)	1 vial	Green	K622-100-5
Triglyceride Standard (1 mM)	0.3 ml	Yellow	K622-100-6

III. Storage and Handling:

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

IV. Reagent Preparation:

TRIGLYCERIDE STANDARD: Freezing for storage may cause the triglyceride standard to separate from the aqueous phase. To re-dissolve the triglyceride standard, keep the cap tightly closed, thaw until liquid then place in a hot water bath (~80-100°C) for 1 minute until the standard look cloudy, then vortex for 30 seconds, the standard should be clear. Repeat the heat and vortex one more time. The triglyceride standard is now completely in solution, and ready for use.

Triglyceride Probe: Dissolve in 220 μl anhydrous DMSO (provided) before use. Store at -20°C, protect from light and moisture. 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.

V. Triglyceride Assay Protocol:

1. Standard Curve Preparation:

For the colorimetric assay, add 0, 2, 4, 6, 8, 10 μl of the 1 mM Triglyceride Standard into wells individually. 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 0.01- 0.1 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.

2. Sample Preparation:* Prepare test samples to a final volume of 50 μl /well with Triglyceride Assay Buffer in a 96-well plate. We suggest testing several dilutions of your sample to make sure the readings are within the standard curve range.

***Notes:** Serum samples can be tested directly. For tissues (100 mg) or cells (10 millions) or other non-aqueous samples, homogenize samples in 1 ml solution containing 5% Triton-X100 in water, slowly heat the samples to 80-100°C in a water bath for 2-5 minutes or until the Triton X-100 becomes cloudy, then slowly cool down to room temperature. Repeat the heating one more time to solublize all triglyceride into solution. Centrifuge for 5 min to remove any insoluble materials. Dilute 10 folds with dH₂O before the 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 addition, so that the assay measure glycerol background only, not triglyceride.

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

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

***Note:** For the fluorometric assay, you may use 10% of the Probe and Enzyme Mix to decrease the background readings, therefore increase detection sensitivity.

5. Mix well. Add 50 μl of the Reaction Mix to each well containing the Triglyceride Standard, test samples and controls. Mix well. Incubate at room temperature for 30-60 minutes-60 minutes gives slightly better result, protect from light.

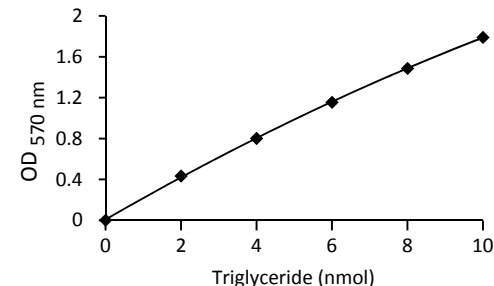
6. Measure O.D. 570 nm for colorimetric assay or Ex/Em = 535/590 nm for fluorometric assay in a microtiter plate reader. The reaction is stable for at least two hours.

7. Calculations: Correct background by subtracting the value derived from the 0 triglyceride standard from all sample readings. Plot the standard curve. Apply sample readings to the standard curve. Triglyceride concentration can then be calculated:

$$C = Ts / Sv \text{ nmol}/\mu\text{l} \text{ or } \mu\text{mol}/\text{ml} \text{ or mM}$$

Where: **Ts** is triglyceride amount from standard curve (nmol).

Sv is the sample volume (before dilution) added in sample wells (μl).



VI. RELATED PRODUCTS:

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| Apoptosis Detection Kits & Reagents | Glucose and Sucrose Assay Kit |
| Cholesterol, LDL/HDL Assay Kits | Glutathione Assay Kits |
| Ethanol and Uric Acid Assay Kits | NAD/NADH and NADP/NADPH Assay Kit |
| Lactate Assay Kit | Pyruvate Assay Kit |