EZScreen™ Triglyceride Quantification Colorimetric Assay Kit (384well)

Rev 04/19

(Catalog # K952-400; 400 assays; Store at -20°C)

I. Introduction:

Triglycerides (TGs) are the main constituent of body fat in humans and animals, as well as vegetable fat. TGs are found as a natural component in human's blood circulatory system. TGs are composed of one molecule of glycerol and 3 molecules of fatty acids. The metabolism of cholesterol generates fatty acids and glycerol, and both can serve as substrates in metabolic pathways to produce energy. Excess TGs can remain in the body as fatty deposits and that may lead to obesity. Elevated triglyceride levels also increase the risk for heart disease, strokes, and type 2 diabetes. BioVision's EZScreen™ Triglyceride Quantification Kit provides a sensitive, easy assay to measure TG concentration in a variety of samples. In the assay, TGs are converted to free fatty acids and glycerol. The released glycerol is then oxidized to generate a product which reacts with a probe to generate a color that can be detected by a spectrophotometer at λ = 590 nm. The method is quantitative, rapid, simple, sensitive, and designed for high throughput format. The kit provides a convenient means for detecting 0.5 to 5 mM Triglycerides in biological samples.

II. Application:

- Measurement of triglycerides in various biological samples
- Mechanistic study of cardiovascular diseases
- Analysis of lipid metabolism

III. Sample Type:

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

IV. Kit Contents:

Components	K952-400	Cap Code	Part Number
Triglyceride Assay Buffer	25 ml	WM	K952-400-1
Triglyceride Probe (in DMSO, anhydrous)	0.8 ml	Red	K952-400-2A
Lipase (lyophilized)	1 vial	Blue	K952-400-3
Triglyceride Enzyme Mix (lyophilized)	1 vial	Green	K952-400-4
Triglyceride Standard (1 mM)	300 µl	Yellow	K952-400-5

V. User Supplied Reagents and Equipment:

- 384-well clear plate with flat bottom
- Multi-well spectrophotometer with 384-well plate reading capability

VI. Storage and Handling:

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

VII. Reagent Preparation and Storage Conditions:

- **Triglyceride Assay Buffer:** Ready to use. Warm to RT before use. Store at -20°C or 4°C.
- **Triglyceride Probe:** Thaw the probe solution by placing it at 37°C water bath for 1-5 min. When in use, leave it at room temperature, protected from light. Store it at -20°C, protected from light, Aliquot to avoid multiple freeze thaws. Use within two months.
- **Lipase:** Resuspend the lyophilized lipase in 880 µl of Triglyceride Assay Buffer. Aliquot and store at -20°C. Use within two months.
- **Triglyceride Enzyme Mix:** Resuspend the lyophilized Triglyceride Enzyme Mix in 880 µl of Triglyceride Assay Buffer. Aliquot and store at -20°C. Use within two months.
- **Triglyceride Standard:** When stored frozen, Triglyceride Standard may separate from the aqueous phase of the solution. To re-dissolve it in the solution, place the tightly closed vial of the Triglyceride Standard in a hot water bath (~80°C) for 1 min or until the standard looks cloudy, then vortex for 30 sec. The standard should become clear. If not, repeat the heat and vortex steps. Once the Triglyceride Standard is completely dissolved in the solution it will become clear and ready to use.

VIII. Triglyceride Assay Protocol:

1. **Sample Preparation:** For serum: add 2 µl of serum samples to a 384-well clear plate. Adjust the volume to 15 µl/well with Triglyceride Assay Buffer. A background control should be performed by replacing 2 µl Lipase with 2 µl Triglyceride Assay Buffer (see section 3). For tissue (~100 mg), cells (~1 million) or other non-aqueous samples, homogenize in 1 ml solution containing 5 % NP-40 in water, slowly heat the samples to 80°C in a water bath for 2-5 min. or until the NP-40 solution becomes cloudy, then cool down to room temperature. Repeat the heating step once more to solubilize all triglycerides. Centrifuge (10000X g, 10 min, 4°C) to remove any insoluble material. Dilute if required with dH_2O before the assay

   Note:

   Normal range of Triglycerides in serum from healthy people is 0.1-1.7 mM and that can be tested directly. Serum sanmples from patients with high levels of triglycerides can be as high as 5 mM. Therefore, to ensure that the readings are within the Standard Curve Range it is recommended to do a 1:2 dilution with triglyceride assay buffer and then adding 1 or 2 µl of the diluted sample to the well.

2. **Standard Curve Preparation:** Dilute 50 µl of 1 mM Triglyceride into 50 µl of Triglyceride Assay Buffer, mix to generate 0.5 mM Triglyceride Standard. Add 0, 2, 4, 6, 8, and 10 µl of the 0.5 mM Triglyceride Standard into a series of wells. Adjust volume to 15 µl/well with Triglyceride Assay Buffer to generate 0, 1, 2, 3, 4, 5 nmol/ well of Triglyceride Standard.

3. **Lipase:** Add 2 µl of Lipase to each Standard and Sample wells. Mix and incubate 40 min at RT to convert triglycerides into glycerol and fatty acids.

   Note: If Samples contain endogenous amount of glycerol, it is recommended of doing a Sample Background Control in order to know the correct amount of glycerol resulted from triglycerides breakdown. For that, Sample is treated with Triglyceride Reaction Mix and probe only, omitting the lipase treatment and Triglyceride Assay Buffer is added to the well(s).

4. **Triglyceride Reaction Mix:** Mix enough reagent for the number of assays to be performed: For each well, prepare a total 10 µl Reaction Mix:

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	Reaction Mix
Triglyceride Assay Buffer	7.5 µl
Triglyceride Probe	2.0 µl
Triglyceride Enzyme Mix	0.5 µl

   Add 10 µl of the Reaction Mix to each well containing the Triglyceride Standard, Samples and Background Control(s). Mix well. Incubate at RT for 60 min, protected from light.

5. **Measurement:** Measure the absorbance in a microplate reader at 590 nm.

6. **Calculations:** Subtract 0 Standard reading from all readings. These are the corrected OD-readings for all Standards. If Sample Background Control reading is significant then subtract the Sample Background Control reading from Sample reading. Plot the TG Standard Curve. For 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

Triglyceride molecular weight: 885.4 g/mol

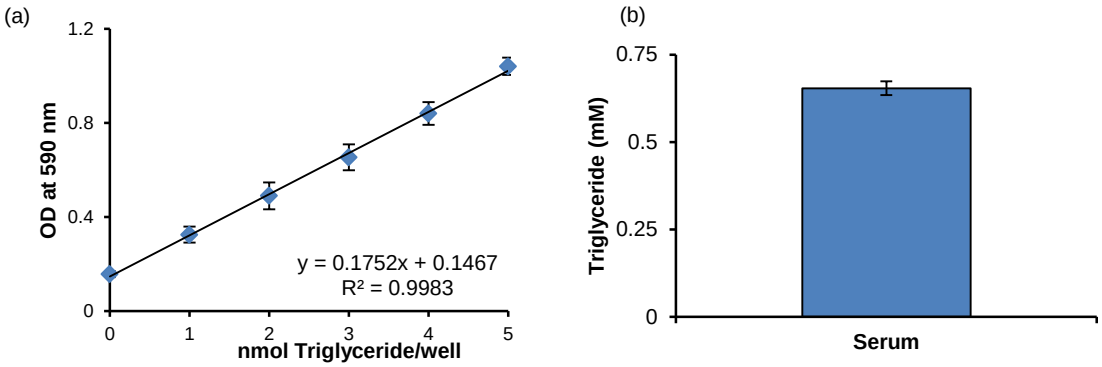

Figure: (a) Triglyceride Standard Curve. (b) Determination of Triglyceride in pooled normal human serum. Undiluted serum sample (2 µl) was assayed directly (no prior treatment) according to the kit protocol. Calculated concentration of triglyceride: 0.65 ± 0.02 mM; (58 ± 1.8 mg/dl).

IX. RELATED PRODUCTS:

PicoProbe™ Triglyceride Quantification Kit (K614)
Cholesterol/Cholesteryl Ester Quantitation Kit (K603)
CETP Activity Fluorometric Assay Kit (K601)
PLTP Activity Fluorometric Assay Kit (K604)
EZScreen™ Glucose Colorimetric Assay Kit-384 Well Format (K950)
Cholesterol/Cholesteryl Ester Quantitation Assay Kit II (K623)
HDL and LDL/VLDL Quantification Kit (K613)
CETP Inhibitor Drug Screening Kit (Fluorometric) (K602)
PLTP Inhibitor Drug Screening Kit (Fluorometric) (K605)
EZScreen™ L-Lactate Colorimetric Assay Kit-384 Well (K951)

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