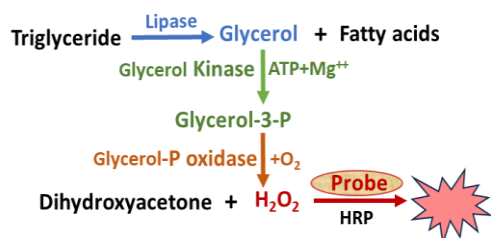


**DESCRIPTION**

Triglycerides (TG) are esters of glycerol and three fatty acids. They are bound to proteins and transported as macromolecular particles called lipoproteins. Triglyceride concentrations are useful in the diagnosis of diabetes mellitus, pancreatitis, heart disease, and other diseases. High triglyceride levels increase the risk of heart disease, strokes, and type-2 diabetes.

Tribioscience’s Triglyceride Fluorometric Assay kit is designed to be a robust, simple, and high throughput method based on enzymatic hydrolysis of triglycerides. The released glycerol is then oxidized to generate hydrogen peroxide, which is catalyzed and reacts with the probe measured by the fluorometric method at Ex/Em 530/590 nm.

**ASSAY PRINCIPLE**



**APPLICATIONS**

**Direct Assays:** As low as 1 mg/dL of Triglyceride in serum, cell culture, tissues and other biological samples.

**KIT CONTENTS**

Name	Size (100 tests)
Triglyceride standard (200 mg/dL)	150 µL
Enzyme Mix (10X)	800 µL
Triglyceride assay buffer	12 mL
Triglyceride Probe	80 µL
Lipoprotein lipase	60 µL

**STORAGE AND HANDLING**

Store kit at -20°C. Shelf life of three months.

**PRECAUTIONS**

Except Enzymes, all the components are warmed up to room temperature before use. Briefly centrifuge all small vials prior to opening.

**ASSAY PROTOCOL**

Briefly place the triglyceride standard tube in a hot water bath (80-100°C) and vortex to make it completely dissolved in solution.

**1. Sample Preparation:**

Serum and plasma can be tested directly. Tissue and cells can be homogenized in 5% NP-40 solution in water. Heat to 80-100°C in a water bath for 2-5 minutes, cool down to room temperature. Repeat the heating one more time to solubilize all triglyceride. Centrifuge for 2 minutes at top speed of a microcentrifuge to remove all insoluble materials. Dilute the supernatant with assay buffer and add 10 µL/well of the samples.

**2. Standard Curve Preparations:**

Tubes	Addition (µL)	Assay Buffer(µL)	Triglyceride Concentration (mg/dL)
1	50 µL Stock	50 µL	100
2	50 µL of Tube#1	50 µL	50
3	50 µL of Tube#2	50 µL	25
4	50 µL of Tube#3	50 µL	12.5
5	50 µL of Tube#4	50 µL	6.25
6	50 µL of Tube#5	50 µL	3.12
7	50 µL of Tube#6	50 µL	1.56
8	0	50 µL	0

Add 10 µL/well of the standards, or sample, or blank control. Use black plate for fluorometric assay.

**3. Addition of Lipoprotein lipase**

For 100 assays, dilute 50 µL of Lipoprotein lipase with 950 µL of assay buffer, add 10 µL to the wells. For sample glycerol background, do not add lipase to the wells, add 10 µL/well of assay buffer instead. Incubate at 37°C for 30 minutes with gentle agitation.

**4. Triglyceride Reaction Mix:** Prepare enough mix for 100 tests as the table below and mix well.

Triglyceride Reaction Mix	Volume
Triglyceride assay buffer	7.20 mL
Enzyme Mix (10X)	800 µL
Triglyceride Probe	60 µL

Add 80 µL of the Reaction Mix to each well containing the Triglyceride Standard and test samples and sample glycerol background control. Tap plate lightly to mix.

**4. Incubation:** Incubate at 37°C for 60 minutes with gentle shaking and protected from light.

**5. Measurement:** Measure at Ex/Em = 530/590 nm for fluorometric assay.

**6. Calculation:** Correct background by subtracting the value of the 0 Triglyceride standard (blank) from all standard readings. Plot the value against standard concentration. Determine the slope using linear regression fitting.

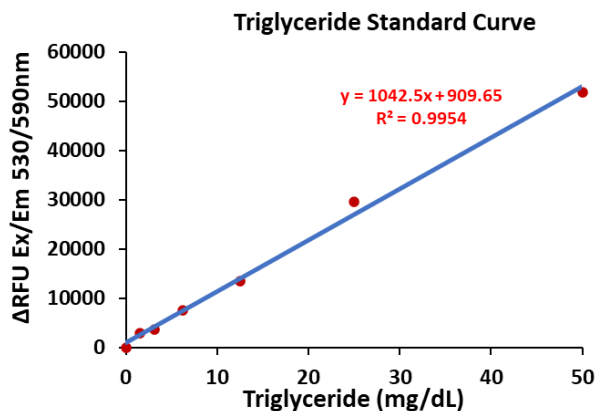
Typical standard curve is  $y = Ax + B$ , A is the slope; B is the y-intercept; X is concentration.

**Triglyceride =  $N * [(RFU_{sample} - RFU_{blank}) - B] / A$  (mg/dL)**

RFU<sub>SAMPLE</sub> and RFU<sub>blank</sub> are fluorescence values of the sample and buffer. N is dilution factor. (Note: If unknown sample results over standard curve range, dilute sample with assay buffer. Repeat the assay; multiply the results by the dilution factor N.)

If the sample glycerol background control is significant, subtract it from sample readings.

**Typical standard curve of triglyceride:**



**RELATED PRODUCTS**

- Triglyceride Colorimetric Assay (TBS2205C)
- Glycerol Colorimetric Assay (TBS2204C)
- Glycerol Fluorometric Assay (TBS2204F)
- Pyruvate Colorimetric Assay (TBS2023C)
- Pyruvate Fluorometric Assay (TBS2023F)
- LDH Cytotoxicity Assay (TBS2002)
- ATP Colorimetric/Fluorometric Assay (TBS2010)
- ADP Colorimetric/Fluorometric Assay (TBS2020)
- Cell Count Kit -8 (TBS2022)
- XTT Cell Viability Assay (TBS2021)
- Caspase-3 Colorimetric Assay (TBS2030)
- Thiol Fluorometric Assay (TBS2026)
- GSH Assay (TBS2028)
- Homocysteine Fluorometric Assay (TBS2091)
- NNMT Inhibitor Screening Assay (TBS2097)
- NNMT Activity Fluorometric Assay (TBS2098)
- G6PDH Activity Colorimetric Assay (TBS2102)
- Cytochrome c Reductase Activity Assay (TBS2116)

**RESEARCH USE ONLY**