（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K238

Specification：96T (Can detect 92 samples without duplication)

Measuring instrument：Microplate reader, biochemical analyzer

Detection range：0.14-9.5 mmol/L

Elabscience® Triglyceride (TG) Colorimetric Assay Kit

(Single Reagent, GPO-PAP Method)

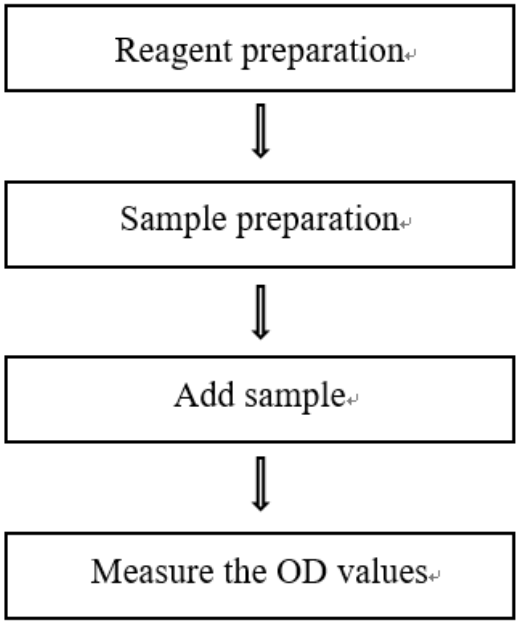
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary

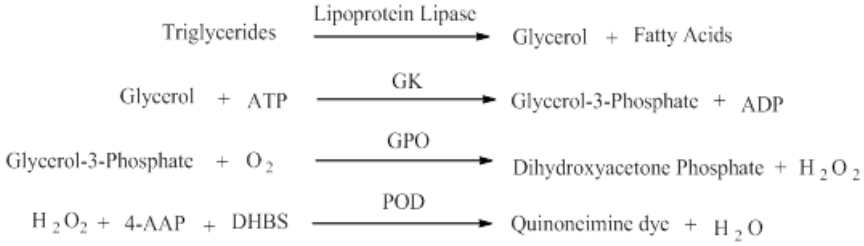


Intended use

This kit applies the GPO-PAP method and it can be used for in vitro determination of triglyceride (TG) content in serum, plasma, cells, culture supernatant and other samples.

Detection principle

Triglycerides (TG) can be hydrolyzed by lipoprotein lipase into glycerol and free fatty acids. Glycerol produces glycerol-3-phosphate and ADP under the catalysis of glycerol kinase (GK). Glycerol-3-phosphate produces hydrogen peroxide under the action of glycerol phosphate oxidase (GPO). In the presence of 4-aminoantipyrine and phenol, hydrogen peroxide is catalyzed by peroxidase (POD) to produce quinones which is proportional to the content of TG.



Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Enzyme Working Solution	25 mL ×1 vial	2-8℃, 12 months, shading light
Reagent 2	2.26 mmol/L Glycerinum Standard	0.1 mL ×1 vial	2-8℃, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (510 nm) or Biochemical analyzer (510 nm), Micropipettor, Water bath, Incubator, Vortex mixer, Centrifuge

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

Reagent preparation

Equilibrate all reagents to room temperature before use.

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Culture supernatant sample: Collect the culture supernatant, centrifuge at 1000 g for 10 min, and take the supernatant for detection

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL homogenate medium with a dounce homogenizer at 4℃.

④ Centrifuge at 12000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Note:

① If the tissue sample is not a high-fat sample, the homogenate medium should be normal saline or PBS.

② If the tissue sample is high-fat sample or partly high lipid sample, the homogenate medium should be absolute alcohol.

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 300-500 μL normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 12000×g for 10 min at 4℃ to remove insoluble material. Collect

supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	1
Mouse serum	1
Rat plasma	1
HepG2 cells (6.5 gprot/L)	1
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse heart tissue homogenate	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① Prevent the formulation of bubbles when adding the liquid to the microplate.

② Protect the reagent from contamination of glucose, cholesterol, etc.

③ The amount of reagent and sample can be increased and decreased as the ratio of 1:100 according to the requirement of automatic biochemical analyzer.

Operating steps

Operate with microplate reader			
	Blank well	Standard well	Sample well
Double distilled water (μL)	2.5		
Standard (μL)		2.5	
Sample (μL)			2.5
Working solution (μL)	250	250	250
Mix thoroughly, incubate at 37℃ for 10 min, measure the OD value at 510 nm with microplate reader.			

Operate with automatic biochemical analyzer	
Sample volume/ Double distilled water (μL)	2.5
Working solution (μL)	250
Incubate at 37℃ for 10 min, set zero with distilled water + working solution, measure the absorbance value A at 510 nm.	
Main wavelength (nm)	510
Reaction type	Endpoint method
Reaction direction	(+)

Calculation

The sample:

1. Serum (plasma) and other liquid sample:

$$\underset{(\text{mmol/L})}{\text{TG}} = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

2. Tissue and cells samples:

$$\underset{(\text{mmol/gprot})}{\text{TG}} = \frac{\Delta A_1}{\Delta A_2} \times c \times f \div C_{pr}$$

[Note]

ΔA_1: $OD_{Sample} - OD_{Blank}$ (If Operate with automatic biochemical analyzer, the OD_{Blank} is 0)

ΔA_2: $OD_{Standard} - OD_{Blank}$ (If Operate with automatic biochemical analyzer, the OD_{Blank} is 0)

c: Concentration of standard.

f: Dilution factor of sample before test.

Cpr: Concentration of protein in sample (gprot/L).

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	1.50	5.00	7.50
%CV	4.4	3.5	4.4

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	1.50	5.00	7.50
%CV	9.0	9.3	9.4

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 105%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mmol/L)	0.35	5.3	9.2
Observed Conc. (mmol/L)	0.4	5.7	9.4
Recovery rate (%)	105	108	102

Sensitivity

The analytical sensitivity of the assay is 0.14 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix II Example Analysis

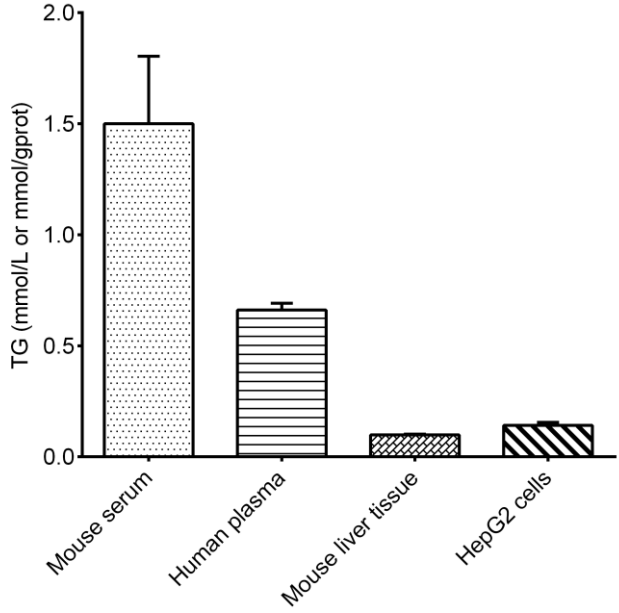
Example analysis:

Take 2.5 μL of mouse serum sample and carry the assay with microplate reader according to the operation table. The results are as follows:

The average OD value of the sample is 0.195, the average OD value of the standard is 0.250, the average OD value of the blank is 0.088, and the calculation result is:

$$\text{TG (mmol/L)} = \frac{0.195\text{-}0.088}{0.250\text{-}0.088} \times 2.26 = 1.49 \text{ mmol/L}$$

Detect mouse serum, human plasma, 10% mouse liver tissue homogenate (the concentration of protein is 10.62 gprot/L) and HepG2 cells (the concentration of protein is 6.50 gprot/L) according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.