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

Catalog No：E-BC-K261-S

Specification：100Assays(96 samples)

Measuring instrument：Spectrophotometer（510 nm）

Detection range：0.19-8.0 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
Tell: 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.

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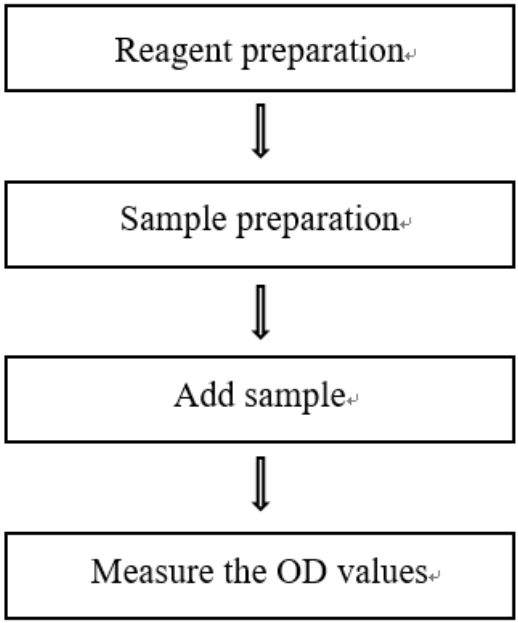
Assay summary

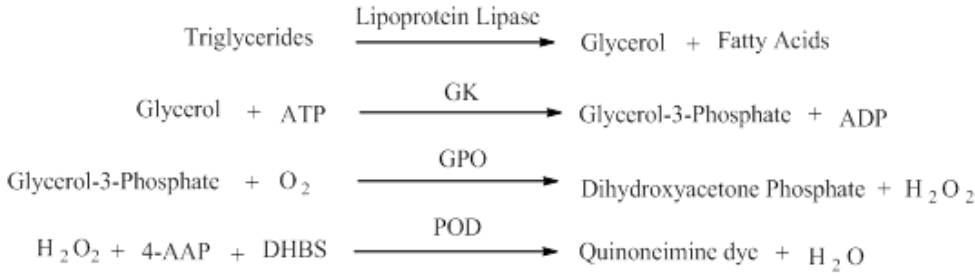


Intended use

This kit applies the GPO-PAP method and it can be used for in vitro determination of triglyceride (TG) content in serum, plasma, and tissue samples, but not for the determination of TG in urine 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	100 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

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:

Spectrophotometer (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), Anhydrous ethanol

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.

Tissue sample:

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

② Wash tissue in cold normal saline (0.9% NaCl).

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

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

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
10% Rat liver tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Mouse brain tissue homogenate	1

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

The key points of the assay

Use the clean EP tubes to prevent contamination of glycerin, glucose and other reagents.

Operating steps

① Blank tube: Take 10 μL of double distilled water to the 2 mL EP tube.
Standard tube: Take 10 μL of 2.26 mmol/L glycerinum standard to the 2 mL EP tube.
Sample tube: Take 10 μL of sample to the 2 mL EP tube.

② Add 1000 μL of enzyme working solution to each tube.

③ Incubate at 37 ℃ for 10 min. Set the spectrometer to zero with double distilled water and measure the OD values of each tube at 510 nm with 0.5 cm optical path cuvette.

Calculation

The sample:

1. Serum (plasma) sample:

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

2. Tissue sample:

$$\underset{(\mu\text{mol/g wet weight})}{\text{TG}} = \frac{\Delta A_1}{\Delta A_2} \times c \times f \div \frac{m}{V}$$

[Note]

ΔA_1: $OD_{Sample} - OD_{Blank}$

ΔA_2: $OD_{Standard} - OD_{Blank}$

c: Concentration of standard.

f: Dilution factor of sample before test.

m: the weight of tissue sample, g.

V: the volume of the homogenate of tissue samples, mL.

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)	0.66	2.50	6.40
%CV	2.7	2.3	2.2

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)	0.66	2.50	6.40
%CV	6.2	6.8	7.1

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 94%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mmol/L)	0.85	3.4	7.2
Observed Conc. (mmol/L)	0.8	3.2	6.6
Recovery rate (%)	98	93	91

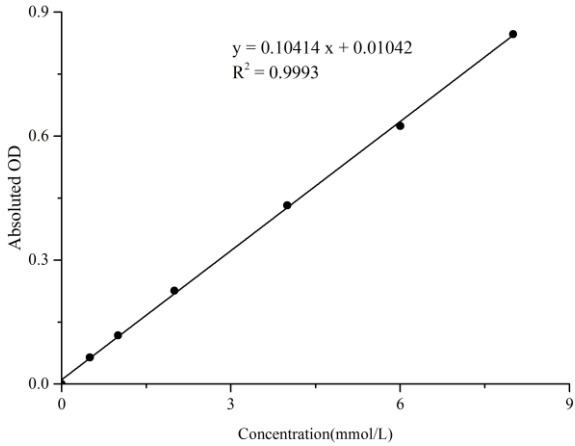
Sensitivity

The analytical sensitivity of the assay is 0.019 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.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (mmol/L)	**0**	**1**	**2**	**4**	**5**	**6**	**8**
Average OD	0.075	0.140	0.193	0.301	0.508	0.700	0.922
Absoluted OD	0.000	0.065	0.118	0.226	0.433	0.625	0.847



Appendix II Example Analysis

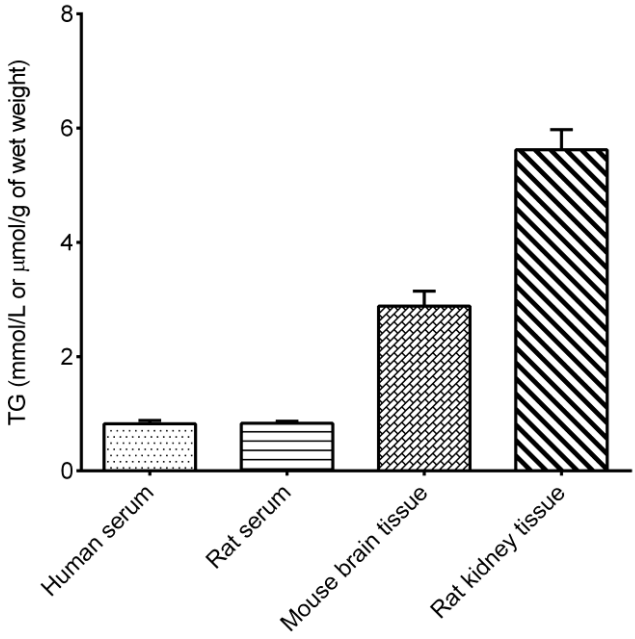
Example analysis:

Take 10 μL of human serum and carry the assay according to the operation steps. The results are as follows:

The average OD value of the sample is 0.130, the average OD value of the standard is 0.271, the average OD value of the blank is 0.050, and the calculation result is:

$$\text{TG (mmol/L)} = \frac{0.130 - 0.050.}{0.271 - 0.050} \times 2.26 \text{ mmol/L} = 0.82 \text{ mmol/L}$$

Detect human serum, rat serum, 10% mouse brain tissue homogenate and 10% rat kidney tissue homogenate 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.