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

Catalog No：E-BC-K261-M

Specification：96T(92 samples)/96T×5(476 samples)

Measuring instrument：Microplate reader (510 nm)

Detection range：0.14-10 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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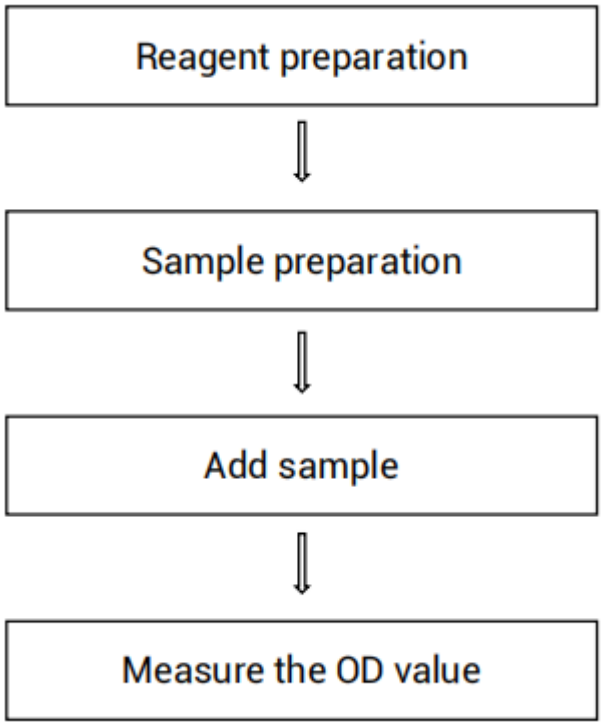
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Assay summary

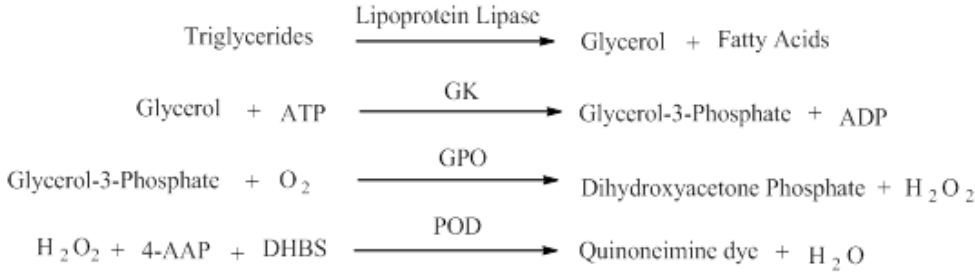


Intended use

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.

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 trinder's reagent, hydrogen peroxide is catalyzed by peroxidase to produce quinones which is proportional to the content of TG.



Kit components & storage

Item	Component	Size 1(96 T)	Size 2(96T×5)	Storage
Reagent 1	Enzyme Working Solution	25 mL × 1 vial	50 mL × 3 vials	2-8°C, 12 months shading light
Reagent 2	2.26 mmol/L Glycerinum Standard	0.1 mL × 1 vial	0.1 mL × 5 vials	2-8°C, 12 months shading light
	Microplate	96 wells	96 × 5 wells	No requirement
	Plate Sealer	2 pieces	7 pieces	
	Sample Layout Sheet	1 piece	7 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), 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（25°C） 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°C 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°C.

④ Centrifuge at 10000×g for 10 minutes 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% 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 serum and plasma; 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

① Prevent the formulation of bubbles when adding the liquid to the microplate.
② Protect the reagent from contamination of glucose, cholesterol, etc.
③ When measuring low content samples , the volume of sample should be increased to 5-10 μL, and the volume of blank well and standard well should be increased at the same time.

Operating steps

① Blank well: Add 2.5 μL of double distilled water to the wells..
Standard well: Add 2.5 μL of 2.26 mmol/L glycerinum standard to the wells.
Sample well:Add 2.5 μL of sample to the wells.
② Add 250 μL of enzyme working solution to each well.
③ Incubate at 37ºC for 10 min, then measure the OD values of each well with microplate reader at 510 nm.

Note: The reagent should be added to the bottom of microplate slowly to avoid gas bubble.

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 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, 2.26 mmol/L.

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)	0.50	2.60	7.40
%CV	4.6	4.1	3.6

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.50	2.60	7.40
%CV	9.1	8.5	10.0

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)	1.6	3.7	8.5
Observed Conc. (mmol/L)	1.7	3.8	9.3
Recovery rate(%)	104	102	109

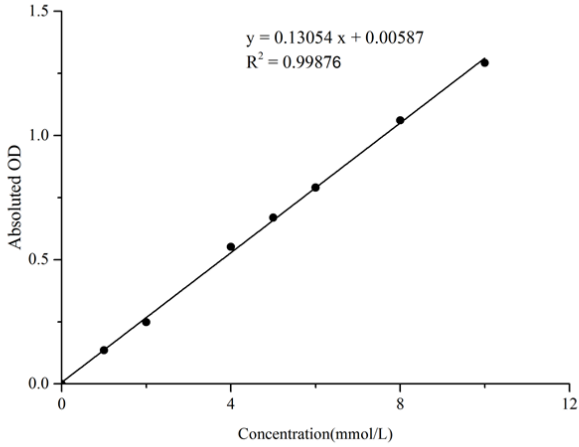
Sensitivity

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.

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	10
Average OD	0.084	0.219	0.332	0.635	0.752	0.874	1.144	1.375
Absoluted OD	0	0.135	0.248	0.551	0.669	0.790	1.060	1.292



Appendix II Example Analysis

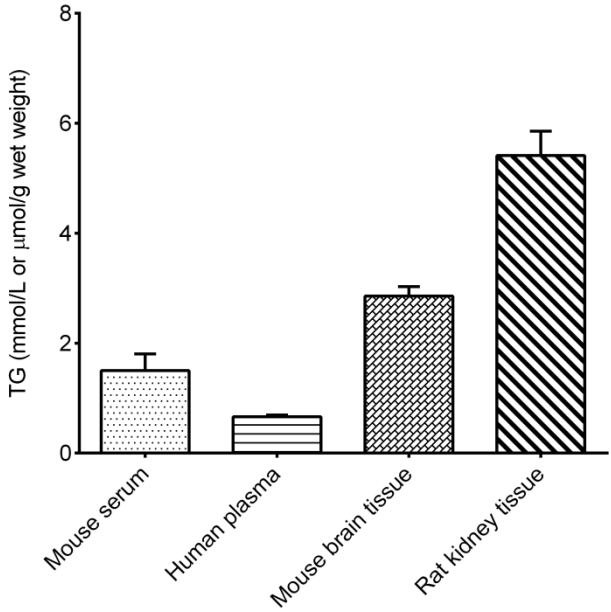
Example analysis：

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

The average OD value of the sample is 0.314, the average OD value of the standard is 0.407, the average OD value of the blank is 0.080, and the calculation result is:

$$\underset{(\text{mmol/L})}{\text{TG}} = \frac{0.314-0.080}{0.407-0.080} \times 2.26 = 1.62 \text{ mmol/L}$$

Detect mouse serum, human plasma, 10% mouse brain tissue homogenate and 10% rat kidney tissue homogenate according to the protocol, the result is as follows：



Appendix III Publications

1. Zhang Y, Qiao Y, Li Z, et al. Intestinal NSD2 aggravates nonalcoholic steatohepatitis through histone modifications[J]. Advanced Science, 2024, 11(33): 2402551.
2. Sun Y, Zhu B, Cong P, et al. Regulating fat globule structure of infant formula based on MFGM to promote lipid uptake by improving lipolysis[J]. Food Hydrocolloids, 2024, 155: 110167.
3. Li Y, Zhou E, Yu Y, et al. Butyrate attenuates cold-induced hypertension via gut microbiota and activation of brown adipose tissue[J]. Science of The Total Environment, 2024, 943: 173835.
4. Liu D, Zhan J, Wang S, et al. Chrysanthemum morifolium attenuates metabolic and alcohol-associated liver disease via gut microbiota and PPARα/γ activation[J]. Phytomedicine, 2024, 130: 155774.
5. Wang Y, Wang J, Zhou T, et al. Investigating the potential mechanism and therapeutic effects of SLXG for cholesterol gallstone treatment[J]. Phytomedicine, 2024, 132: 155886.
6. Wang M L, Zhang Y J, He D L, et al. Inhibition of PLA2G4A attenuated valproic acid-induced lysosomal membrane permeabilization and restored impaired autophagic flux: Implications for hepatotoxicity[J]. Biochemical Pharmacology, 2024, 227: 116438.

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.