Triglyceride (TG) Colorimetric Assay Kit (Single Reagent, GPO-PAP Method)

Catalog No: E-BC-K261-M

Method: Colorimetric method

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

Instrument: Microplate reader

Sensitivity: 0.14 mmol/L

Detection range: 0.14-10 mmol/L

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

General information

▲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, and tissue samples.

▲Background

TG is the main component of vegetable oil, animal fat, low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL), and serves as a carrier and source of energy for fatty acids. Triglyceride turnover rate determines the utilization of fatty acids in mammalian tissues. Any dysfunction in this process may lead to changes in glucose metabolism, insulin resistance and type 2 diabetes.

▲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.

$$\text{Glycerin trilaurate} + \text{H20} \xrightarrow{\text{Lipase}} \text{Glycerinum} + \text{Fatty acid}$$

$$\text{Glycerinum} + \text{ATP} \xrightarrow{\text{Glycerol Kinase}} \text{Glycerol} - 3 - \text{phosphoric acid} + \text{ADP}$$

$$\text{Glycerol} - 3 - \text{phosphoric acid} + \text{O2} \xrightarrow{\text{Glycerol}-3-\text{Phosphate Oxidase}} \text{Hydroxyacetone phosphate} + \text{H2O2}$$

$$\text{Parachlorphenol} + \text{H2O2} + 4 - \text{AAP} \xrightarrow{\text{Peroxidase}} \text{Red quinone}$$

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Enzyme Working Solution	25 mL×1 vial	2-8℃，6 months, shading light
Reagent 2	2.26 mmol/L Glycerinum Standard	0.1 mL × 1 vial	2-8℃，6 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.

▲Materials prepared by users

Instruments

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

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 5 mL)

Reagents

Double distilled water, Isopropanol (AR)， Normal saline (0.9% NaCl),

PBS (0.01 M, pH 7.4)

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲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 such as cells, 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.

Pre-assay preparation

▲Reagent preparation

Bring all reagents to room temperature before use.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.14-10 mmol/L).

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
HepG2 cells	1

Note: The diluent of serum (plasma) is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4); The diluent of animal tissue and cells is isopropanol.

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	510 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85
B	B	B	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86
C	S1	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
D	S2	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
E	S3	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
F	S4	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
G	S5	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
H	S6	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92

Note: A, blank wells; B, standard wells; S1-S92, sample wells.

▲Operation table

1. Blank well: Take 2.5 μL of double distilled water to the wells.

   Standard well: Take 2.5 μL of reagent 2 to the wells.

   Sample well: Take 2.5 μL of sample to the wells.
2. Add 250 μL of reagent 1 into the wells of step 1.
3. Incubate at 37 ºC for 10 min, then measure the OD values of each well with microplate reader at 510 nm.

▲Operation table

	Blank well	Standard well	Sample well
Double distilled water (μL)	2.5		
Reagent 2 (μL)		2.5	
Sample (μL)			2.5
Reagent 1 (μL)	250	250	250
Mix thoroughly, incubate at 37°C for 10 min, measure the OD value at 510 nm with microplate reader.			

▲Calculation

1. Serum (plasma) sample and other liquid sample:

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

2. Tissue sample:

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

3. Cells sample:

$$TG(\mu mol/10^6) = \frac{\Delta A_1}{\Delta A_2} \times c \times f \div (N / V)$$

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 isopropanol, mL.

N: the number of cells. For example, the number of cells is $5*10^6$, N is 5.

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	0.14-10 mmol/L	Average intra-assay CV (%)	4.1
Sensitivity	0.14 mmol/L	Average inter-assay CV (%)	9.2
Average recovery rate (%)	105		

▲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 0.314, 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:

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

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲Serum

Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4°C . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80°C for a month.

▲Plasma

Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g for 10 min at 4°C . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

▲Tissue

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃ . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of isopropanol (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4℃ . Take the supernatant to preserve it on ice for detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

▲Cells

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (2×10^6): homogenization medium (μL) =1: 200. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80℃ for a month.

Note:

1. Homogenized medium: Isopropanol (AR)

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.
Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

(3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/time, interval for 3 s, the total time is 5 min).

▲ Notes for sample

1. Please predict the concentration before assaying. If the sample concentration is out of the detection range, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.