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

Catalog No：E-BC-F033

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：0.8-30 μmol/L

Elabscience®Triglyceride(TG) Fluorometric Assay Kit

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.

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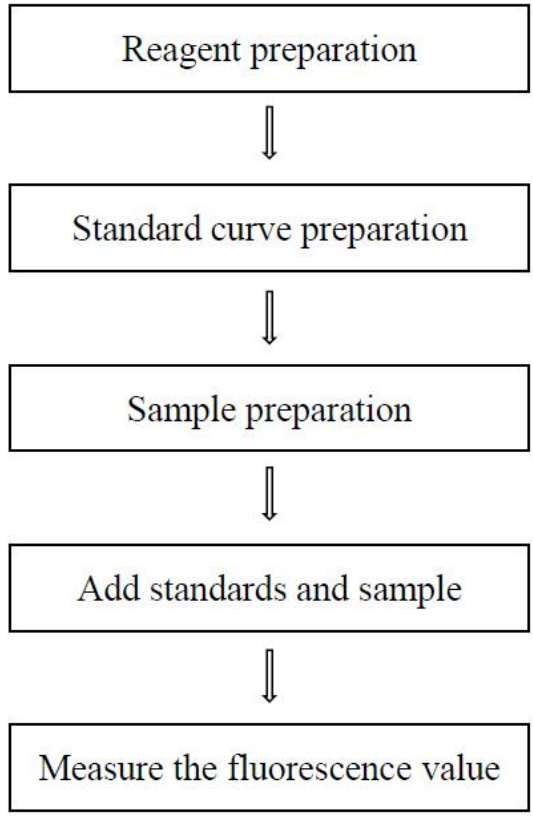
Assay summary



Intended use

This kit can be used for the determination of triglyceride in serum, plasma, tissue and cell samples.

Detection principle

Triglyceride is converted by enzyme to produce hydrolytic products, which is catalyzed by enzymes to produce fluorescent substances. The content of triglyceride in samples can be calculate by measuring the fluorescence value.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Enzyme Working Solution	12 mL ×1 vial	25 mL × 1 vial	2-8°C, 12 months shading light
Reagent 2	Extraction Solution	50 mL ×1 vial	50 mL × 2 vials	2-8°C, 12 months
Reagent 3	Probe	0.3 mL ×1 vial	0.5 mL × 1 vial	-20°C, 12 months shading light
Reagent 4	1 mmol/L Standard Solution	0.5 mL ×1 vial	0.5 mL × 1 vial	2-8°C, 12 months
	Black 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:

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Centrifuge, Incubator （37°C）

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The Preparation of chromogenic working solution：

For each well, prepare 200 uL of chromogenic working solution (mix well 196 μL of enzyme working solution and 4 μL of probe). The chromogenic working solution should be prepared on spot and protected from light.

③ The preparation of 30 μmol/L standard solution:

Dilute 30 μL of 1 mmol/L standard solution with 970 μL of extraction solution, mix well. Store at 2-8°C for 3 days.

④ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 30 μmol/L standard solution with extraction solution to a serial concentration. The recommended dilution gradient is as follows: 0, 6, 12, 18, 24, 30 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥
Concentration (μmol/L)	**0**	**6**	**12**	**18**	**24**	**30**
30 μmol/L standard (μL)	0	40	80	120	160	200
Extraction solution (μL)	200	160	120	80	40	0

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 PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL extraction solution with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min 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).

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 200 μL extraction solution with a ultrasonic cell disruptor at 4°C.

④ Centrifuge at 10000×g for 10 min 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
10% Porcine heart tissue homogenate	3-6
10% Porcine liver tissue homogenate	8-10
1×10^6 CHO cells	1-2
1×10^6 293T cells	1-2
Human serum	100-120
Mouse serum	100-120

Note: The diluent is extraction solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

The preparation equipment needs to be cleaned several times before the preparation of the chromogenic working solution to prevent contamination by impurities.

Operating steps

① Standard well: add 20 μL of standard with different concentrations into the corresponding well.

Sample well: add 20 μL of sample into the wells.

② Add 200 μL of chromogenic working solution into each well.

③ Mix fully, incubated at 37°C for 5 min. Measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean fluorescence value of the blank (Standard #①) from all standard readings. This is the absoluted fluorescence value.

3. Plot the standard curve by using absoluted fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

$$\text{TG content (mmol/L)} = \frac{\Delta F - b}{a} \times f \div 1000^{*}$$

2. Tissue and cell sample:

$$\text{TG content (μmol/gprot)} = \frac{\Delta F - b}{a} \div C_{pr} \times f$$

[Note]

ΔF: Absolute fluorescence intensity of sample ($F_{Sample} - F_{Blank}$).

C_{pr}: The concentration of protein in sample, gprot/L.

1000*: 1000 μmol =1 mmol.

f: Dilution factor of sample before tested.

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 (μmol/L)	2.50	15.00	25.00
%CV	4.2	3.8	3.7

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 (μmol/L)	2.50	15.00	25.00
%CV	10.0	8.9	8.4

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%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	7	15	22
Observed Conc. (μmol/L)	7.5	15.8	22.7
Recovery rate (%)	107	105	103

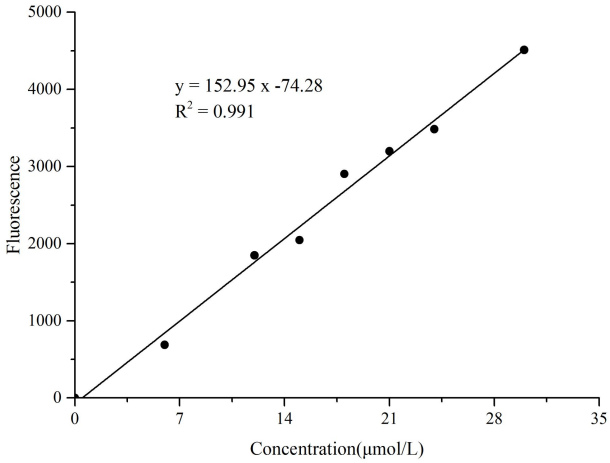
Sensitivity

The analytical sensitivity of the assay is 0.8 μmol/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 fluorescence 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 (μmol/L)	**0**	**6**	**12**	**15**	**18**	**21**	**24**	**30**
Fluorescence value	1364	2032	3049	3402	4057	4330	4874	5703
	1267	1975	3280	3320	4382	4698	4724	5951
Average fluorescence value	1316	2003	3164	3361	4220	4514	4799	5827
Absoluted fluorescence value	0	687	1849	2045	2904	3198	3483	4511



Appendix II Example Analysis

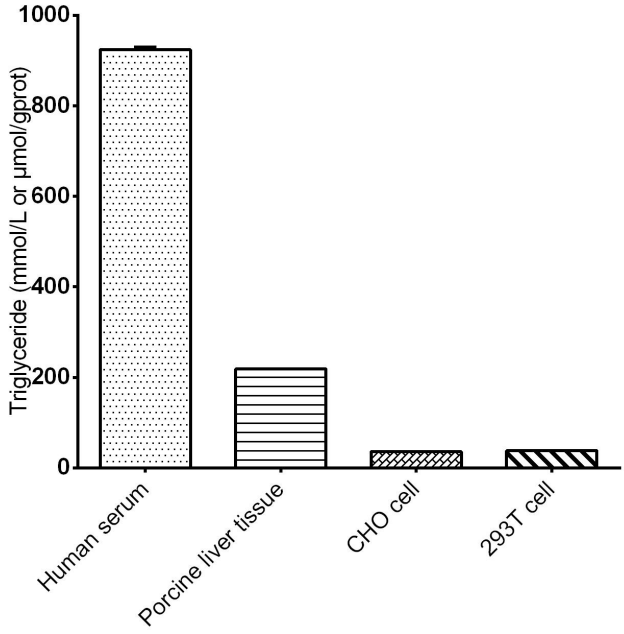
Example analysis：

Dilute the human serum for 100times and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 152.95 x – 74.284, the average fluorescence value of the sample is 2664, the average fluorescence value of the control is 1316, and the calculation result is:

TG content (mmol/L)= (2664 -1316 + 74.284) ÷ 152.95 × 100 ÷ 1000 = 0.93 mmol/L

Detect Human serum (dilute for 100 times) , 10% Porcine liver tissue homogenate (the concentration of protein is 5.67 gprot/L, dilute for 5 times), 1×10^6 CHO cells (the concentration of protein is 0.98 gprot/L), 1×10^6 293T cells (the concentration of protein is 1.21 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.