TECHNICAL MANUAL

Triglyceride Fluorometric Assay Kit

- **SKU CODE:** MAES0257
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Measure fluorescence intensity

2. Intended use

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

3. Detection principle

Triglyceride is enzymatically converted to produce hydrolytic products, which are then catalyzed by enzymes to produce fluorescent substances. The triglyceride content in samples can be calculated by measuring the fluorescence value.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Enzyme Working Solution	12 mL x 1 vial	25 mL x 1 vial	2-8 °C, 12 months, shading light
Reagent 2	Extraction Solution	50 mL x 1 vial	50 mL x 2 vials	2-8 °C, 12 months
Reagent 3	Probe	0.3 mL x 1 vial	0.5 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 4	1 mmol/L Standard Solution	0.5 mL x 1 vial	0.5 mL x 1 vial	2-8 °C, 12 months
	Black Microplate	96 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

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

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of chromogenic working solution:** For each well, prepare 200 µL of chromogenic working solution (mix 196 µL of enzyme working solution with 4 µL of probe thoroughly). The chromogenic working solution should be prepared immediately before use and protected from light.
3. **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 up to 3 days.
4. **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.

7. Sample preparation

1. **Sample preparation:** Serum and plasma: Detect directly. If not assayed on the same day, serum or plasma can be stored at -80 °C for one 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 10,000 xg for 10 min to remove insoluble material. Collect supernatant and keep on ice for detection. 5. Meanwhile, determine the protein concentration of supernatant (MAES0177). Cell (adherent or suspension) samples: 1. Harvest the number of cells needed for each assay (initial recommendation 1x10^6 cells). 2. Wash cells with PBS (0.01 M, pH 7.4). 3. Homogenize 1x10^6 cells in 200 µL extraction solution with an ultrasonic cell disruptor at 4 °C. 4. Centrifuge at 10,000 xg for 10 min to remove insoluble material. Collect supernatant and keep on ice for detection. 5. Meanwhile, determine the protein concentration of supernatant (MAES0177).
2. **Dilution of sample:** The recommended dilution factor for different samples is as follows (for reference only): 10% Porcine heart tissue homogenate: 3-6 10% Porcine liver tissue homogenate: 8-10 1x10^6 CHO cells: 1-2 1x10^6 293T cells: 1-2 Human serum: 100-120 Mouse serum: 100-120 Note: The diluent is extraction

solution. For dilution of other sample types, perform a pretest to confirm the dilution factor.

8. The key points of the assay

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

9. Operating steps

1. Standard wells: Add 20 µL of standard with different concentrations into the corresponding wells. Sample wells: Add 20 µL of sample into the wells.
2. Add 200 µL of chromogenic working solution into each well.
3. Mix thoroughly and incubate at 37 °C for 5 min. Measure the fluorescence intensity of each well at excitation wavelength of 535 nm and emission wavelength of 587 nm.

10. 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 absolute fluorescence value.

3. Plot the standard curve using absolute fluorescence value of standards 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:

TG content (mmol/L) = (deltaF - b)/a x f ÷ 1000

2. Tissue and cell sample:

TG content (µmol/gprot) = (deltaF - b)/a ÷ C_{pr} x f

[Note]

deltaF: 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 testing.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision

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.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (µmol/L)	2.50	15.00	25.00
%CV	10.0	8.9	8.4

Recovery

Three samples of high, medium, and low concentration were tested with 6 parallel determinations for each concentration to obtain an average recovery rate of 105%.

Recovery

	Standard 1	Standard 2	Standard 3
Expected Conc. (µmol/L)	7	15	22
Observed Conc. (µmol/L)	7.5	15.8	22.7

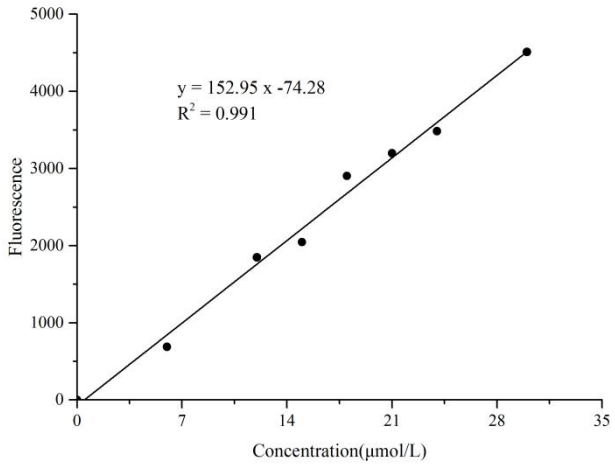
	Standard 1	Standard 2	Standard 3
Recovery rate (%)	107	105	103

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.

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), the standard curve and data are provided below for reference only:



Standard curve

Concentration (µmol/L)	Fluorescence value		Average fluorescence value	Absolute fluorescence value
0	1364	1267	1316	0
6	2032	1975	2003	687
12	3049	3280	3164	1849
15	3402	3320	3361	2045

Concentration (μmol/L)	Fluorescence value		Average fluorescence value	Absolute fluorescence value
18	4057	4382	4220	2904
21	4330	4698	4514	3198
24	4874	4724	4799	3483
30	5703	5951	5827	4511

12. Appendix II Example Analysis

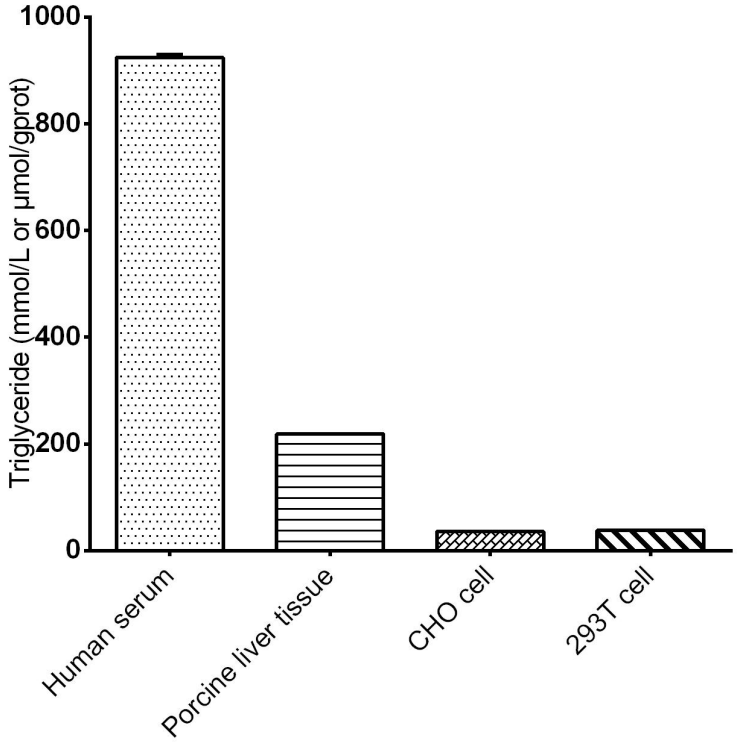
Example analysis:

Dilute the human serum 100-fold and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 152.95x - 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 (diluted 100-fold), 10% Porcine liver tissue homogenate (protein concentration is 5.67 gprot/L, diluted 5-fold), 1×10^6 CHO cells (protein concentration is 0.98 gprot/L), 1×10^6 293T cells (protein concentration is 1.21 gprot/L) according to the protocol. The results are as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie will not be responsible for any arising problems or legal responsibilities caused by using the kit for clinical diagnosis or other purposes.

2. Please read the instructions carefully and calibrate the instruments before performing experiments. Please follow the instructions strictly during the experiments.

3. Protective measures must be taken by wearing lab coat and latex gloves.

4. If the concentration of the substance is not within the detection range, an additional dilution or concentration step should be performed on the sample.

5. It is recommended to perform a pretest if your sample is not listed in the instruction manual.

6. The experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption caused by using the assay kits. It is advisable to calculate the expected sample usage and reserve sufficient samples before use.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.