TECHNICAL MANUAL

Free Fatty Acids (FFA) Fluorometric Assay Kit

- **SKU CODE:** MAES0010
- **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
- Incubate with enzyme solution
- Add chromogenic agent
- Measure fluorescence intensity

2. Intended use

This kit can be used to measure the non-esterified free fatty acids (FFA) content in serum, plasma, animal tissue and cell samples.

3. Detection principle

Free fatty acids produce acyl coenzyme A in the presence of acyl synthase, which produces hydrogen peroxide in the presence of acyl oxidase. In the presence of the enzyme and probe, hydrogen peroxide reacts to produce the fluorescence substrate. The fluorescence intensity at the excitation wavelength of 535 nm and emission wavelength of 590 nm is directly proportional to the concentration of free fatty acids.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	50 mL x 1 vial	50 mL x 2 vials	-20 °C, 12 months
Reagent 2	Substrate	0.06 mL x 1 vial	0.12 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 3	Enzyme Reagent 1	Powder x 1 vial	Powder x 2 vials	-20 °C, 12 months, shading light
Reagent 4	Enzyme Reagent 2	Powder x 1 vial	Powder x 2 vials	-20 °C, 12 months, shading light
Reagent 5	Scavenger	0.6 mL x 1 vial	1.2 mL x 1 vial	-20 °C, 12 months

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 6	1 mmol/L Standard Solution	0.4 mL x 1 vial	0.4 mL x 1 vial	-20 °C, 12 months
	Black Microplate	96 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Micropipette, Vortex mixer, Centrifuge, Fluorescence microplate reader (Ex/Em=535 nm/590 nm)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of enzyme application solution 1:** Dissolve one vial of enzyme reagent 1 with 3 mL of buffer solution, mix well to dissolve. Store at -20 °C for 1 month protected from light.
3. **Preparation of enzyme application solution 2:** Dissolve one vial of enzyme reagent 2 with 300 µL of buffer solution, mix well to dissolve. Store at -20 °C for 1 month protected from light.
4. **Preparation of chromogenic agent:** For each well, prepare 50 µL of chromogenic agent (mix well 34 µL of buffer solution, 1 µL of substrate, 5 µL of enzyme application solution 2 and 10 µL of scavenger). The chromogenic agent should be prepared fresh and protected from light.
5. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 1 mmol/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 40, 50, 60, 80, 100 µmol/L.

7. Sample preparation

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 buffer solution with a dounce homogenizer at 4 °C.

4. Centrifuge at 12,000 x 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 (MAES0177).

Cell (adherent or suspension) samples:

1. Harvest the number of cells needed for each assay (initial recommendation $2x10^6$ cells).

2. Wash cells with PBS (0.01 M, pH 7.4).

3. Homogenize $2x10^6$ cells in 100 µL buffer solution with an ultrasonic cell disruptor at 4 °C.

4. Centrifuge at 12,000 x 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 (MAES0177).

Dilution of sample:

The recommended dilution factor for different samples is as follows (for reference only):

Human serum: 10-20

Rat serum: 20-40

Mouse plasma: 10-30

Rabbit serum: 10-30

10% Rat liver tissue homogenate: 20-40

10% Mouse kidney tissue homogenate: 20-40

10% Rat brain tissue homogenate: 20-40

10% Rat lung tissue homogenate: 20-40

Note: The diluent is buffer solution. For the dilution of other sample types, please do a pre-test to confirm the dilution factor.

8. The key points of the assay

1. The chromogenic agent should be protected from light.

2. Avoid repeated freezing and thawing of substrate, enzyme application solution 1 and enzyme application solution 2. It is recommended to aliquot them into smaller quantities and store at -20 °C.

9. Operating steps

1. Standard well: Add 10 µL of standard with different concentrations. Sample well: Add 10 µL of samples.
2. Add 50 µL of enzyme application solution 1 into each well.
3. Mix fully with microplate reader for 10 s and incubate at 37 °C for 10 min.
4. Add 50 µL chromogenic agent into each well.
5. Incubate at 37 °C for 10 min.
6. Measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 590 nm with fluorescence microplate reader.

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 by using absolute 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:

FFA (µmol/L) = (deltaF - b) ÷ a x f

2. Tissue and cell sample:

FFA (µmol/g) = (deltaF - b) ÷ a x f ÷ C_{pr}

[Note]

deltaF: Absolute F value of sample (F_{sample} - F_{blank}).

f: Dilution factor of sample before tested.

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

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)

Parameters	Sample 1	Sample 2	Sample 3
Mean (µmol/L)	18.00	40.00	75.00
%CV	4.6	4.3	3.5

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)	18.00	40.00	75.00
%CV	5.2	5.9	4.3

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times in parallel to get the average recovery rate of 98%.

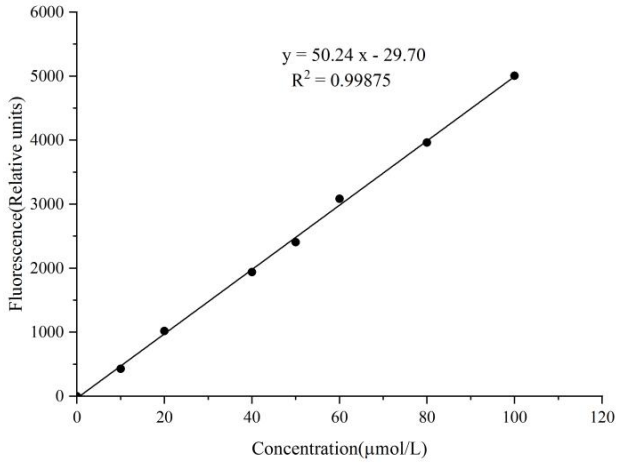
	Standard 1	Standard 2	Standard 3
Expected Conc. (µmol/L)	15	45	70
Observed Conc. (µmol/L)	15.0	45.4	70.2
Recovery rate (%)	99	97	98

Sensitivity

The analytical sensitivity of the assay is 1.20 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 as below for reference only:



Concentration (µmol/L)	Fluorescence value		Average fluorescence value	Absolute fluorescence value
0	333	351	342	0
10	782	761	772	430
20	1351	1374	1362	1020
40	2273	2290	2282	1940
50	2696	2802	2749	2407
60	3346	3506	3426	3084

Concentration (μmol/L)	Fluorescence value		Average fluorescence value	Absolute fluorescence value
80	4261	4349	4305	3963
100	5264	5430	5347	5005

12. Appendix II Example Analysis

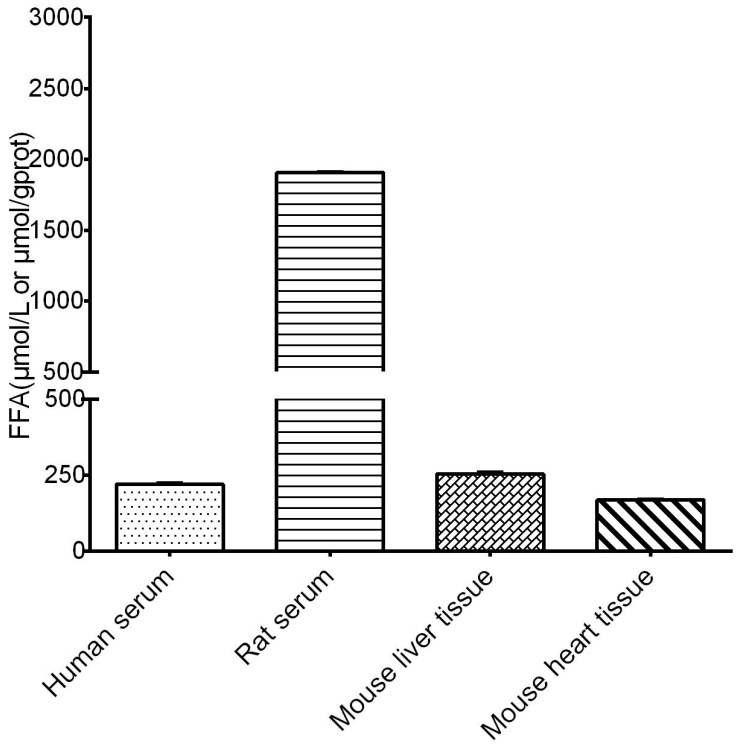
Example analysis:

For human serum, take 10 μL of human serum diluted with buffer solution 20 times and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 50.24x - 29.7, the average OD value of the sample is 1008, the average OD value of the blank is 492, and the calculation result is:

FFA content (μmol/L) = (1008 - 492 + 29.7) ÷ 50.24 x 20 = 217.23 μmol/L

Detect human serum (dilute 20 times), rat serum (dilute 40 times), 10% rat liver tissue homogenate (dilute 30 times) and 10% mouse brain tissue homogenate (dilute 30 times) according to the protocol, the result is as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. 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 resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

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.