Technical Manual

Free Fatty Acids (FFA) Fluorometric Assay Kit

- Catalogue Code: MAES0010
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Fluorimetric method

Specification:

96T

Range:

0.58-20 μmol/L

Sensitivity:

0.58 μmol/L

Storage:

-20°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Free fatty acids, also known as non-esterified fatty acids, are derived from dietary or the metabolism of adipose tissue. In adipose tissue, hormone-sensitive lipase (HSL) decomposes triglycerides to produce glycerol and fatty acids. Circulating in the body with free fatty acids combined with plasma albumin, used as an energy source easily absorbed by muscles, brains, and other tissues and organs. FFA is not only the product of fat hydrolysis, but also the substrate of fat synthesis. The concentration of FFA is related to lipid metabolism, glucose metabolism and endocrine function.

3. Intended Use

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

4. Detection Principle

Free fatty acids produce acyl coenzyme A in the presence of acyl synthase, which produces hydrogen 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 react 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.

5. Kit components & storage

Item	Specification	Storage
Buffer Solution	60 mL × 1 vial	-20°C, 6 months
Substrate	0.12 mL × 1 vial	-20°C, 6 months, avoid direct sunlight
Enzyme Reagent 1	Lyophilized ×1 vial	-20°C, 6 months, avoid direct sunlight
Enzyme Reagent 2	Lyophilized ×1 vial	-20°C, 6 months, avoid direct sunlight
Scavenger	0.2 mL × 1 vial	-20°C, 6 months
Standard Solution (1mmol/L)	0.2 mL × 1 vial	-20°C, 6 months
Extracting Solution	60 mL × 1 vial	-20°C, 6 months, avoid direct sunlight
Black Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Fluorescence microplate reader (Ex/Em=535 nm/590 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

6. Assay Notes:

1. If the sample is serum or plasma, it doesn't need control well.

2. If the sample are tissue, the control well is essential. And it can set one control well if the dilution factor of all tissue samples is the same. Every sample needs a control well if the dilution factor of the tissue samples is different.

3. 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.

7. Reagent Preparation

1. Bring all reagent to room temperature before use.

2. Preparation of **enzyme application solution 1:** Dissolve a vial of enzyme reagent 1 with 1.2 mL of buffer solution and mix fully. The prepared enzyme application solution 1 can be stored at -20°C for 1 month with avoid direct sunlight.

3. Preparation of **enzyme application solution 2:** Dissolve a vial of enzyme reagent 2with 120 μL of buffer solution and mix fully. The prepared enzyme application solution 2 can be stored at -20°C for 1 month with avoid direct sunlight.

4. Preparation of **chromogenic agent:** Mix the buffer solution, substrate, enzyme application solution 2 and scavenger at a ratio of 47:1:1:1. Prepare the fresh solution before use and stored with avoid direct sunlight.

5. Preparation of **standard (20 μmol/L):** Mix the standard solution (1mmol/L) and buffer solution at a ratio of 1:49. Prepare the fresh solution before use.

6. Preparation of extracting application solution (for determination of tissue samples in blank wells): Dilute the extracting solution with buffer solution according to the dilution factor of sample. For example, the tissue sample was diluted for 300 times, so dilute the extracting solution with buffer solution at a ratio of 1:299.

8. Sample Preparation

1. Serum sample:
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.

2. Plasma sample:
Take fresh blood into the tube which has anticoagulant (heparin is recommended), 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.

3. Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of PBS (0.01 M, pH 7.4) (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

4. Cell sample:
Wash the cells with PBS (0.01 M, pH7~7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add PBS at a ratio of cell number (10^6): PBS (μL) =1: 300-500. 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. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (6.43-407 μmol/L).

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

Sample Type:	Dilution Factor
10% Rat liver tissue homogenate	1
10% Rat brain tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Mouse kidney tissue homogenate	1
Jurkart cell supernatant	1-5
Rat serum	1-5
Rat plasma	1
HL-60 cell supernatant	5-15
10% Rat heart tissue homogenate	1
10% Rat stomach tissue homogenate	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4);

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 340 nm

Plate Set Up:

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

Note: A-H, standard wells; S1-S80, sample wells.

10. Operation Steps

The pre-treatment of sample

1. For serum (plasma) and cell culture supernatant samples:
Take 0.05 mL of sample to 2 mL EP tube, add 0.15 mL of protein precipitator and mix fully, centrifuge at 3100 g for 10 min, then take 0.05 mL of the supernatant for detection.

2. For tissue and cell samples:
Take 0.05 mL of tissue or cell homogenate sample to 2 mL EP tube, add 0.15 mL of protein precipitator and mix fully, centrifuge at 3100 g for 10 min, then take 0.05 mL of the supernatant for detection.

The preparation of standard curve

Dilute standard solution (1 mmol/L) with standard diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 25, 50, 100, 200, 300, 400, 450 μmol/L.

The measurement of samples

1. **Standard well:** Add 50 μL of standard solution with different concentrations to the corresponding wells.
   Sample well: Add 50 μL of sample supernatant to the corresponding wells.

2. Add 150 μL of reaction working solution to each well.

3. Mix fully for 5 s with microplate reader and measure the OD values of each well at 340 nm with microplate reader, recorded as A_1.

4. Add 10 μL of enzyme working solution into each well.

5. Mix fully for 5 s with microplate reader and incubate at 37°C for 40 min. Measure the OD values of each well at 340 nm with microplate reader, recorded as A_2. $\Delta A = A_2 - A_1$.

Operation Table

	Standard well	Sample well
Standard solution with different concentrations (μL)	50	
Sample supernatant (μL)		50
Reaction working solution (μL)	150	150
Mix fully for 5 s with microplate reader and measure the OD values of each well at 340 nm with microplate reader, recorded as A_1.		
Enzyme working solution (μL)	60	60
Mix fully for 5 s with microplate reader and incubate at 37°C for 40 min. Measure the OD values of each well at 340 nm with microplate reader, recorded as A_2. $\Delta A = A_2 - A_1$.		

11. Calculations

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. Serum (plasma) and other liquid sample:

Glutamic acid content (μmol/L)
= (ΔA ' - b) ÷ a ×4 × f

2. Tissue and cell sample:

Glutamic acid content (μmol/gprot)
= (ΔA ' - b) ÷ a ×4 × f ÷ C_{pr}

y: $\Delta A_{Standard}$ - ΔA_{Blank} (ΔA_{Blank} is the change of OD value when the standard concentration is 0)

x: The concentration of standard

a: The slope of standard curve

b: The intercept of standard curve

ΔA ' : ΔA ' =ΔA_{Sample} - ΔA_{Blank}

4*: Dilution factor in the step of pretreatment of sample

f: Dilution factor of sample before test

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

12. Performance Characteristics

Detection Range	6.43-407 μmol/L
Sensitivity	6.43 μmol/L
Average recovery rate (%)	98
Average inter-assay CV (%)	8.3
Average intra-assay CV (%)	2.5

Analysis

Take 50 μL of 10% rat liver tissue homogenate supernatant, carry the assay according to the operation table.

The results are as follows:

Standard curve: y = 0.0007 x + 0.0046, the change OD value of the sample is 0.138, the change OD value of the blank is 0.018, the concentration of protein in sample is 8.52 gprot/L, and the calculation result is:

Glutamic acid content (μmol/gprot)

= （0.138-0.018-0.0046）÷ 0.0007 × 4 ÷ 8.52
= 77.39 μmol/gprot

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

Email: info@assaygenie.com **Web:** www.assaygenie.com

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Web: www.assayenie.com