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Free Fatty Acid(FFA) Content Fluorometric Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Fluorometric microplate reader

Catalog Number: AK1042

Size: 50T/48S ; 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact SunLong staff in time

Reagent

50T size

100T size

Storage

Extract solution

Liquid 60 mL×1

Liquid 110 mL×1

2-8℃

Reagent I

Liquid 3.7 mL×1

Liquid 6 mL×1

2-8℃

Reagent II

Powder×1

Powder×1

-20℃

Reagent III

Powder×1

Powder×1

-20℃

Reagent IV

Powder×1

Powder×2

-20℃

Reagent V

Powder×1

Powder×1

-20℃

Reagent VI

Powder×1

Powder×1
-20℃
Reagent VII
Liquid 0.4 mL×1
Liquid 0.6 mL×1
2-8℃
Reagent VIII
Powder×2
Powder×4
-20℃
Standard
Liquid 1 mL×1
Liquid 1 mL×1
-20℃
96-well black opaque plate
Plate×1
Plate×1
-
Sealing plate film (Cat#YA0249)
Film×2
Film×2
-

Solution Preparation:

1. Reagent II: Dissolve in 0.129 mL of distilled water before use. Unused reagents should be aliquoted and stored at-20°C for 8 weeks, avoiding repeated freeze-thaw cycles.
2. Reagent III: Dissolve in 0.125 mL of distilled water before use. Unused reagents should be aliquoted and stored at-20°C for 8 weeks, avoiding repeated freeze-thaw cycles.
3. Reagent IV: Take one vial before use. Dissolve in 0.7 mL of distilled water before use. Unused reagents should be aliquoted and stored at-20°C for 8 weeks, avoiding repeated freeze-thaw cycles.
4. Reagent V: Dissolve in 0.2 mL(50T)/ 0.3 mL(100T) of absolute alcohol before use. Unused reagents should be stored at-20°C for 4 weeks
5. Reagent VI: Dissolve in 0.35 mL(50T)/ 0.6 mL(100T) of distilled water before use. Unused reagents should be aliquoted and stored at 4°C for 4 weeks, avoiding repeated freeze-thaw cycles.
6. Reagent VIII: Add 75 μL of DMSO to one Reagent V before use. Aliquot according to the sample number. It could be stored at room temperature and protected from light for immediate use. The remaining portion could be stored at -20°C for 3 months and protected from light, avoiding repeated freezing and thawing.
7. Working solution I: Prepare according to the sample size in the ratio of Reagent I: Reagent II: Reagent III: Reagent IV=500 μL: 10 μL:10 μL: 100 μL (620 μL, about 10T).
8. Working solution II: Prepare according to the sample size in the ratio of distilled water: Reagent V:

Reagent VI: Reagent VII: Reagent VIII =355 μL: 25 μL:50 μL:50 μL: 20μL (520 μL, about 10T).

9. Standard: 5 mmol/L (5000 nmol/mL) palmitic acid standard solution.

Note:

1. The reagent IV and reagent VIII are freeze-dried reagent, there may be a large difference in the amount of reagents observed by the naked eye or even a small amount of the phenomenon, this phenomenon does not affect the use, the actual quality of the same.

2. If the prepared working solution is insufficient, prepare it in a new reagent bottle; never prepare it in the original reagent bottle containing existing working solution.

Product Description

Free fatty acids (FFA) are non-esterified fatty acids. Free fatty acid is one of the substances that neutral fat decomposes. FFA in serum are primarily intermediate products of lipid metabolism and serve as essential metabolic substrates for cellular energy metabolism, providing energy to organs such as the heart, liver, and skeletal muscle.

In the presence of ATP and Mg^{2+}, acyl-CoA synthetase (ACS) catalyzes the binding of free fatty acids to coenzyme A to form acyl-CoA. Then, acyl-CoA is catalyzed by acyl-CoA oxidase (ACO) to generate hydrogen peroxide (H_2O_2). Under enzymatic action, H_2O_2 reacts with the fluorescent probe to generate a strongly fluorescent product, whose fluorescence intensity at an excitation wavelength of 535 nm and an emission wavelength of 587 nm is proportional to the H_2O_2 concentration. This kit determines the FFA content in samples by measuring the amount of H_2O_2 generated. This kit can be used to analyze serum, cultured cells, and animal tissue samples.

Reagents and Equipment Required but Not Provided:

Fluorometric microplate reader, low temperature centrifuge, pipette, mortar/homogenizer/cell ultrasonic crusher, constant temperature incubator/metal bath, 96-well black opaque plate(provided), sealing plate film(provided), ethanol (>98%, AR), DMSO(>98%， AR), ice and distilled water.

Procedure

I. Sample preparation(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

1. Tissue: Add Extract solution according to the ratio of tissue mass (g) : Extract solution volume (mL) = 1:5 ~10 (it is recommended to weigh 0.1g sample and add 1.0mL Extract solution), after ice bath homogenization, centrifuge at 4℃, 12000 rpm for 10min, collect the supernatant and place it on ice for measurement.

2. Bacteria/cells: According to the number of bacteria/cells (106): the volume of the Extract solution (mL) is 1-5:1 (it is recommended to add 1 mL of the Extract solution to 10 million bacteria/cells), ultrasonic wave breaks bacteria/cells (ice bath, power 200W, ultrasonic 3s, interval 6s, total time 3min), centrifuge at 4℃, 12000 rpm for 10min, collect the supernatant and place it on ice for measurement.

3. Serum (plasma) sample: Detect sample directly. (If the solution is turbid, centrifuge to take the supernatant and then measure)

II. Determination procedure:

1.

Preheat the fluorometric microplate reader for 30min, adjust excitation wavelength/emission

 wavelength to 535nm/587nm.

2.

Before the experiment, work solution I and work solution II were balanced to normal temperature.

3.

Standard dilution: Dilute 5000 nmol/mL palmitic acid standard solution with extract solution to obtain 50, 40, 30, 20, 10, 5, 2.5 nmol/mL standard solution before use.

4.

Add reagents with the following list:

Reagent (μL)	Test tube(T)	Standard tube(S)	Blank tube(B)
Sample	20	-	-
Standard	-	20	-
Distilled water	-	-	20
Working solution I	60	60	60
Mix thoroughly and incubate at 37°C away from light for 10 minutes.			
Working solution II	50	50	-

Mix thoroughly and incubate at 37°C away from light for 10 minutes, measure RFU at Ex/Em= 535nm/587nm, recording as RFUT, RFUS, RFUB. Calculate ΔRFUT= RFUT- RFUB, ΔRFUS= RFUS- RFUB. (The blank tube and standard curve only need to measure 1-2 times.)

Note: The reaction time at 37°C should be accurate. If the sample size is too large, it is recommended to determine in batches.

III. Calculations:

1.

Standard curve

According to the concentration of the standard tube (x, nmol/mL) and the absorbance ΔRFUS (y, ΔRFUS), a standard curve was established. According to the standard curve, ΔRFUT (y, ΔRFUT) was brought into the formula to calculate the sample concentration (x, nmol/mL).

2.

Calculation of FFA content

1) Protein concentration:

FFA content (nmol//mg prot)=x×VE÷(Cpr×VE)×F =x÷Cpr×F

2) Sample mass:

FFA content (nmol/g mass) = x×VE÷ W×F=x÷W×F

3) Bacteria/cells number:

FFA content (nmol/106 cell) =x×VE÷ N×F=x÷N×F

4) Liquid volume :

FFA content (nmol/mL) =x×F

VE: Added extract solution volume, 1mL; W: Sample mass, g; N: The number of bacteria/cells, count by 106; Cpr: Sample protein concentration, mg/mL.

Notes:

1.

For the determination of free fatty acid (FFA) content in plant samples such as leaves, roots, stems, and fruits, the FFA content assay kit with product code AK1042 is recommended.

2.

If the RFUT exceeds the linear range, dilute the sample with extract solution before re-measurement.

For low RFUT, increase the sample volume appropriately, simultaneously modify the calculation formula.

3.

The extract solution is also required when diluting serum or plasma samples.

4.

Differences in the sensitivity of various fluorometric microplate readers may lead to variations in the detected RFU, which is a normal phenomenon. Provided that the reagent preparation and experimental procedure are correct and the standard curve exhibits good linearity, the detection results are considered accurate and valid.

Experimental examples:

1.

Dilute 5000 nmol/mL palmitic acid with Extract solution to obtain 50, 40, 30, 20, 10, 5, 2.5 nmol/mL standard solution. Measure RFU of standard tubes and establish the standard curve y = 241.77x + 5.6631，R2 = 0.9985.

2.

Results of different samples:

Samples

Mass/

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Number
Dilution ratio
RFUT
ΔRFUT
FFA content
Unit
Rubbit Serum
-
10
9049.6
6632.5
274.1 nmol/mL
Rat Liver
0.1000g
80
9485.3
7068.2
23369.4 nmol/g mass
BV2 cell
5×106 cell
1
5160.5
2743.4
2.3 nmol/106 cell
Soy bean
0.1011g
20
10124.0
7706.9
6301.4 nmol/g mass

Note: During sample processing, approximately 0.1 g of rat liver and soybeans were weighed and added to 1 mL of extract solution for homogenization; 5×10^6 BV2 cells were added to 1 mL of extract solution for ultrasonic disruption; rabbit serum was used directly for subsequent processing.