Free Fatty Acids (FFA) Content Assay Kit

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Detection instrument: Spectrophotometer/ Microplate reader

Catalog Number: BC0595

Size: 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 Solarbio staff in time.

Reagent name	Size	Storage
Extract solution	Solution 110 mL×1	2-8℃
Reagent I	Required but not provided	-
Reagent II A	Solution 20 mL×1	2-8℃
Reagent II B	Powder×1	2-8℃
Reagent III	Powder×2	2-8℃
Standard	Powder×1	RT

Solution preparation:

1. **Reagent I:** Before the experiment, take a glass bottle and prepare it in a ratio of n-heptane: anhydrous methanol: chloroform=24:1:25 (Self-supplied reagent). Cover it tightly and mix well. Approximately 50mL is needed and store at 2-8 ℃. Seal it promptly after use.
2. **Reagent II:** Pour Reagent II B into Reagent II A and heat at 40°C for 20 minutes. This solution is a saturated solution. If there is still powder undissolved, just take supernatant and use it. It can be stored at 2-8°C for 3 months;
3. **Reagent III:** Anhydrous ethanol of 13 mL is added to the reagent bottle before use. It can be stored at 2-8°C for 2 weeks.
4. **Standard:** Before use, transfer the powder to a 10 mL glass bottle, add 7.8 mL of chloroform to fully dissolve it, that is, a standard solution of palmitic acid of 5 μmol/mL. The unused reagent is sealed with a sealing film and can be stored at 2-8 °C for 4 weeks.

Product Description:

Free fatty acids (FFA) is both a product of fat hydrolysis and a substrate for fat synthesis. The concentration of FFA in serum is related to lipid metabolism, glucose metabolism, and endocrine function.

FFA combines with copper ions to form fatty acid copper salts, which dissolve in chloroform; By using the copper reagent method to determine the copper ion content, the free fatty acid content can be calculated.

Technical Specifications:

The detection limit: 0.039 μmol/mL

The linear range: 0.05-2 μmol/mL

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Required material:

Spectrophotometer/Microplate reader, centrifuge, adjustable pipette, vortex mixer, micro glass cuvette/96 well plate, mortar/homogenizer, one 50mL glass bottle, one 10mL glass bottle, n-heptane (>98%，AR), anhydrous methanol (>98%，AR), chloroform (>98%，AR), anhydrous ethanol (>98%，AR), ice and distilled water.

Procedure:

I. Sample Extraction:

1. Serum sample:

   Leave the blood at room temperature for 1 hour, and then centrifuge at 3500rpm for 15 minutes at 4℃. Aspirate the serum from the upper layer and store on ice until testing .

2. Tissue sample:

   After the tissue was rinsed with normal saline, the surface water was absorbed with absorbent paper. According to the ratio of tissue mass (g): extract volume (mL) of 1:5~10 (it is recommended to weigh about 0.1g of tissue and add 1mL of extract solution), perform ice bath homogenization. Centrifuge at 8000 rpm for 10 min at 4°C and take the supernatant for testing.

II. Determination procedure:

1. Preheat the spectrophotometer/microplate reader 30 minutes, adjust the wavelength to 550 nm and set zero with **anhydrous ethanol**.
2. Preheat Reagent II in 37℃ water bath for 20 minutes.
3. Prepare standard working solution: Dilute the standard solution with **chloroform** to 1, 0.8, 0.6, 0.4, 0.2, 0.1, 0.05 μmol/mL.
4. Standard dilution table

Number	Pre dilution concentration (μmol/mL)	Standard solution volume (μL)	Chloroform volume (μL)	Post dilution concentration (μmol/mL)
1	5	200	800	1
2	1	160	40	0.8
3	1	120	80	0.6
4	1	160	240	0.4
5	0.4	200	200	0.2
6	0.2	200	200	0.1
7	0.1	200	200	0.05

Note: Each standard tube in the following experiment requires 30 μL of standard solution (be careful not to directly test the absorbance of the standard solution in this step).

5. Add reagents into 1.5 mL centrifuge tube with the following list

Reagent name (μL)	Control tube(C)	Test tube (T)	Blank control (B)	Standard tube(S)
Distilled water	30	-	-	-

After shaking for 5 minutes, let stand for 15min. Take 0.2mL in a micro glass cuvette/96-well plate. The absorbance value is measured at 550 nm. The absorbance is recorded as A_C, A_T, A_B and A_S. ΔA_S

Sample	-	30	-	-
Chloroform	-	-	30	-
Standard	-	-	-	30
Reagent I	300	300	300	300
Reagent II	120	120	120	120
Shake for 15min fully, centrifuge at 3000 rpm for 10 minutes.				
Supernatant	50	50	50	50
Reagent III	200	200	200	200

$=A_S-A_B$, $\Delta A_T=A_T-A_C$.

Note: Control tube and Blank control and standard curve only need do once or twice.

III. Calculation:

1. Create standard curve
   According to the concentration of the standard tube (x, μmol/mL) and the absorbance ΔAs (y, ΔA_S), establish a standard curve. According to the standard curve, bring the ΔA assay (y, ΔA_T) into the formula to calculate the sample concentration (x, μmol/mL).
2. Serum FFA content
   FFA content (μmol/L)=1000x
3. Tissue FFA content
   1) Calculated by sample protein concentration
   FFA content (μmol/mg prot)=x ×Vsv÷ (Cpr×Vsv)=x ÷ Cpr
   2) Calculated by sample weight
   FFA content (μmol/g weight)=x × Vsv÷ W
   Vsv: Total supernatant volume,1 mL
   Cpr: Supernatant protein concentration, mg/mL;
   W: Sample weight, g;
   1 L=1000 mL.

Note:

1. Reagent III should be prepared as late as possible, and it can be prepared after adding Reagent II.
2. It is necessary to ensure that the oscillation frequency and time of each tube are consistent.
3. Experiment should be completed the measurement within 30 minutes.
4. The upper solution should not be added directly to the 96-well-plates, and the solution should be sealed and discarded after test.
5. Because most of the reagents used are organic solvents, repeated absorption of the same suction head will result in inaccurate volume. It is recommended to replace the tips when absorb the different

reagents.

Experimental example:

1. Take the mouse serum for sample processing, according to the determination steps, using 96 well plate to measure A_C = 0.098, A_T = 0.308, $\Delta A_T = A_T - A_C$= 0.308-0.098 = 0.21, bring into the standard curve: y = 0.6679x + 0.019, calculate x = 0.286
   FFA content (μmol/L) = 1000x = 1000×0.286 = 286 μmol/L.

Recent Products Citations:

[1] Ye Q, Liu Y, Zhang G, Deng H, Wang X, Tuo L, Chen C, Pan X, Wu K, Fan J, Pan Q, Wang K, Huang A, Tang N. Deficiency of gluconeogenic enzyme PCK1 promotes metabolic-associated fatty liver disease through PI3K/AKT/PDGF axis activation in male mice. Nat Commun. 2023 Mar 14;14(1):1402. doi: 10.1038/s41467-023-37142-3. PMID: 36918564; PMCID: PMC10015095.

[2] Wei X, Yin F, Wu M, Xie Q, Zhao X, Zhu C, Xie R, Chen C, Liu M, Wang X, Ren R, Kang G, Zhu C, Cong J, Wang H, Wang X. G protein-coupled receptor 35 attenuates nonalcoholic steatohepatitis by reprogramming cholesterol homeostasis in hepatocytes. Acta Pharm Sin B. 2023 Mar;13(3):1128-1144. doi: 10.1016/j.apsb.2022.10.011. Epub 2022 Oct 13. PMID: 36970193; PMCID: PMC10031266.

[3] Liu Z, Zhang L, Qian C, Zhou Y, Yu Q, Yuan J, Lv Y, Zhang L, Chang X, Li Y, Liu Y. Recurrent hypoglycemia increases hepatic gluconeogenesis without affecting glycogen metabolism or systemic lipolysis in rat. Metabolism. 2022 Nov;136:155310. doi: 10.1016/j.metabol.2022.155310. Epub 2022 Sep 3. PMID: 36063868

[4] Cai B, Ma M, Zhang J, Wang Z, Kong S, Zhou Z, Lian L, Zhang J, Li J, Wang Y, Li H, Zhang X, Nie Q. LncEDCH1 improves mitochondrial function to reduce muscle atrophy by interacting with SERCA2. Mol Ther Nucleic Acids. 2021 Dec 10;27:319-334. doi: 10.1016/j.omtn.2021.12.004. PMID: 35024244; PMCID: PMC8717430.

[5] Cai B, Ma M, Zhang J, Wang Z, Kong S, Zhou Z, Lian L, Zhang J, Li J, Wang Y, Li H, Zhang X, Nie Q. LncEDCH1 improves mitochondrial function to reduce muscle atrophy by interacting with SERCA2. Mol Ther Nucleic Acids. 2021 Dec 10;27:319-334. doi: 10.1016/j.omtn.2021.12.004. PMID: 35024244; PMCID: PMC8717430.

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[1] Laurell S, Tibbling G. Colorimetric micro-determination of free fatty acids in plasma[J]. Clinica chimica acta, 1967, 16(1): 57-62.

[2] Itaya K. A more sensitive and stable colorimetric determination of free fatty acids in blood[J]. Journal of lipid Research, 1977, 18(5): 663-665.

[3] Duncombe W G. The colorimetric micro-determination of non-esterified fatty acids in plasma[J]. Clinica chimica acta, 1964, 9: 122-125.

Related Products:

BC2340/BC2345	Lipase(LPS) Activity Assay Kit
BC1080/BC1085	Alcohol Dehydrogenase(ADH) Activity Assay Kit

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