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

Catalog Number: BC0590

Size: 50T/48S

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 60 mL×1	2-8°C
Reagent I	Required but not provided	-
Reagent II A	Solution 16 mL×1	2-8°C
Reagent II B	Powder×1	2-8°C
Reagent III	Powder×2	2-8°C
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 °C. 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 32 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 four 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 linear range: 0.025-0.8 μmol/mL

The detection limit: 0.012 μmol/mL

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Required material:

Spectrophotometer, centrifuge, adjustable pipette, 1mL glass cuvette, vortex mixer, mortar/homogenizer, one 50mL glass bottle, one 10mL glass bottle, n-heptane (>98%， AR), anhydrous

Chloroform	-	-	50	-
Standard	-	-	-	50

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°C. 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 30 minutes, adjust the wavelength to 550 nm and set zero with **anhydrous ethanol**.
2. Preheat Reagent II in 37°C water bath for 20 minutes.
3. Dilution of the standard: Dilute the standard with **chloroform** to 0.8, 0.6, 0.4, 0.2, 0.1, 0.05, 0.025 μmol/mL.
4. Standard dilution table

Serial Number	Pre dilution concentration (μmol/mL)	Standard volume (μL)	**Chloroform volume** (μL)	Diluted 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 50 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	50	-	-	-
Sample	-	50	-	-

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Reagent I	500	500	500	500
Reagent II	200	200	200	200
Shake for 15min fully, centrifuge at 3000 rpm for 10 minutes.				
Supernatant	200	200	200	200
Reagent III	800	800	800	800

After shaking for 5 minutes, let stand for 15min. The absorbance value is measured at 550 nm. The absorbance is recorded as A_C, A_T, A_B and A_S. $\Delta A_S = A_S - A_B$, $\Delta A_T = A_T - A_C$.

Note: The control tube, blank tube, and standard curve only need to be done 1-2 times.

III. Calculation:

1. Create standard curve

   According to the concentration of the standard tube (x, μmol/mL) and the absorbance ΔA_S (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 fresh weight)=x × Vsv÷ W

   Vsv: Total supernatant volume,1 mL

   Cpr: Supernatant protein concentration, mg/mL；

   W: Sample weight, g;

   1000: Unit conversion factor, 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. The operating parameters of each sample must be consistent.
3. Try to complete the measurement within 30 minutes and seal it properly before discarding it.
4. 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 = 0.091, A_T = 0.527, $\Delta A = A_T - A_C$= 0.527-0.091=0.436, bring into the standard curve: y

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= 1.3429x+0.1189, calculate x = 0.236.

FFA content (μmol/L) = 1000x = 1000×0.236=236 μmol/L.

Recent Products Citations:

[1] Hu C, Xin Z, Sun X, Hu Y, Zhang C, Yan R, Wang Y, Lu M, Huang J, Du X, Xing B, Liu X. Activation of ACLY by SEC63 deploys metabolic reprogramming to facilitate hepatocellular carcinoma metastasis upon endoplasmic reticulum stress. J Exp Clin Cancer Res. 2023 May 1;42(1):108. doi: 10.1186/s13046-023-02656-7. PMID: 37122003; PMCID: PMC10150531.

[2] Gao J, Song YJ, Wang H, Zhao BR, Wang XW. Mindin Activates Autophagy for Lipid Utilization and Facilitates White Spot Syndrome Virus Infection in Shrimp. mBio. 2023 Apr 25;14(2):e0291922. doi: 10.1128/mbio.02919-22. Epub 2023 Feb 13. PMID: 36779788; PMCID: PMC10127999.

[3] Zhang B, Zhang Z, Song D, Lyu X, Zhao W. Reduction of intestinal fat digestion and absorption by β-glucan secreted by Rhizobium pusense via interference in triglyceride hydrolysis. Food Funct. 2022 Oct 17;13(20):10802-10810. doi: 10.1039/d2fo01123a. PMID: 36193692.

[4] Ye X, Sun P, Lao S, Wen M, Zheng R, Lin Y, Gan L, Fan X, Wang P, Li Z, Yan X, Zhao L. Fgf21-Dubosiella axis mediates the protective effects of exercise against NAFLD development. Life Sci. 2023 Dec 1;334:122231. doi: 10.1016/j.lfs.2023.122231. Epub 2023 Nov 5. PMID: 37935276.

[5] Zhou Q, Kong D, Li W, Shi Z, Liu Y, Sun R, Ma X, Qiu C, Liu Z, Hou Y, Jiang J. LncRNA HOXB-AS3 binding to PTBP1 protein regulates lipid metabolism by targeting SREBP1 in endometrioid carcinoma. Life Sci. 2023 May 1;320:121512. doi: 10.1016/j.lfs.2023.121512. Epub 2023 Feb 27. PMID: 36858312.

References:

[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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