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Free Fatty Acids (FFA) Content Assay Kit

Note: Take two or three different samples for prediction before test.

Detection instrument: Spectrophotometer/ Microplate reader

Cat No: AK0535

Size: 100T/96S

Components:

Extract solution: 100 mL×1. Storage at room temperature.

Reagent I: This solution needs to be prepared by the customer.

The solution is prepared according to the ratio of n-heptane: anhydrous methanol: chloroform=24:1:25 (provide for oneself) and put in a glass bottle before the experiment. Mix well after capping, store at room temperature.

Reagent II: 20 mL×1. Storage at room temperature.

Reagent III: Powder×2. Storage at 4℃ . Working solution: 13 mL of anhydrous ethanol is added to the reagent bottle before use, fully dissolved and preserved for one week at 4℃ .

Standard: Powder ×1, 10 mg of palmitic acid. Storage at room temperature. Standard working solution (5 μmol/mL): before use, the reagent is transferred to a 10 mL of glass bottle and fully dissolved with 7.8 mL of chloroform.

Product Description:

Free fatty acids (FFA) is the product of fat hydrolysis, also the substrate of fat synthesis. The concentration of FFA in serum is related to lipid metabolism, glucose metabolism and endocrine function. FFA combines with copper ion to form fatty acid copper salt which is dissolved in chloroform. The content of free fatty acids can be calculated by copper reagent method.

Required material:

Mortar, ice, desk centrifuge, pipette, visible spectrophotometer/microplate reader, micro glass cuvette/96-well flat-bottom plates, 25 mL glass bottle, 15 mL glass bottle, n-heptane, anhydrous methanol, chloroform, anhydrous ethanol and distilled water.

Procedure:

I. Sample Extraction:

1. Serum sample: Leave the blood at room temperature for 1 hour, and then centrifuge at 4℃ and 3500 rpm for 15 minutes. The upper serum is stored in a refrigerator at -20℃ for test.

2. Tissue sample: After the tissues are washed with saline, the surface water is absorbed with absorbent paper. It is suggested to add 1 mL of Extract solution when 0.1 g is taken. After homogenization, the supernatant is obtained by centrifugation at 8000 rpm for 10 minutes at 4℃, take the supernatant and placed on ice for test.

II. Determination procedure:

1. Preheat the spectrophotometer/microplate reader 30 minutes, adjust the wavelength to 550 nm and set zero with distilled water.

2. Preheat Reagent Ⅱ in 37℃ water bath for 30 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. 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	-	-	-
Sample	-	30	-	-
Chloroform	-	-	30	
Standard	-	-	-	30
Reagent I	300			
Reagent II	120			
Mixed thoroughly, centrifuge at 3000 rpm for 10 minutes.				
Supernatant	50			
Reagent III	200			

After 2 minutes of full oscillation, it is rested for 15 minutes. The absorbance is measured at 550 nm with 0.2 mL in micro glass cuvette or/96-well flat-bottom plates. The absorbance is recorded as A_C, A_T, A_B and A_S, respectively.

Note: Control tube and Blank control can just do once.

III. Calculation:

1. Create standard curve

   Taking the concentration of standard solution as the x-axis and the A standard tube (A=A standard tube-A blank tube) as the y-axis, the standard curve is drawn and the equation y=kx+b is obtained. ΔA ($\Delta A=A_T-A_C$) is introduced into the equation to obtain x.

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 fresh 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;

   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. Mark sure complete the measurement within 30 minutes
4. The upper solution should not be added directly to the 96-well flat-bottom plates, and 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, $\Delta A = A_T - A_C$ = 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] Shanming Ruan, Zhiqian Zhang, Xinxin Tian, et al. Compound Fuling Granule Suppresses Ovarian Cancer Development and Progression by disrupting mitochondrial function, galactose and fatty acid metabolism. Journal of Cancer. September 2018;(IF3. 182)

[2] Tunyu Jian, Yuexian Wu, Xiaoqin Ding, et al. A novel sesquiterpene glycoside from Loquat leaf alleviates oleic acid-induced steatosis and oxidative stress in HepG2 cells. Biomedicine & Pharmacotherapy. January 2018;(IF3.743)

[3] Rui Wang, Junhua Yuan, Caishun Zhang, et al. Neuropeptide Y-Positive Neurons in the Dorsomedial Hypothalamus Are Involved in the Anorexic Effect of Angptl9. Frontiers in Immunology. December 2018;(IF3.72)

[4] Yao L, Chen S, Li W. Fatostatin inhibits the development of endometrial carcinoma in endometrial carcinoma cells and a xenograft model by targeting lipid metabolism[J]. Archives of Biochemistry and Biophysics, 2020: 108327.

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.

Technical Specifications:

The linear range: 0.05-2 μmol/mL

The detection limit: 0.0390 μmol/mL