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Free Fatty Acid (FFA) Content Assay Kit (Copper Soap Method)

Free Fatty Acid (FFA) Content Assay Kit (copper soap method)

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

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: AK1041-100T-96S

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 SunLong staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Self-supplied reagent	-
Reagent I	Liquid 25 mL×1	2-8℃
Standard	Powder×1	2-8℃

Solution Preparation:

1. Extract solution: Self-provided n-hexane or isooctane, about 100 mL, sealed and saved in time after use; a 30 mL brown reagent bottle is provided in the kit, which is only used for packaging. Please mark the name of the reagent yourself. (Preferential use of n-hexane is recommended).

2. Standard: The reagent was placed in a brown glass bottle in the reagent bottle. Before use, 5.86 mL of the Extract solution was added to fully dissolve and prepare a standard substance of 10μmol/mL for later use. The unfinished reagent was sealed with a sealing film and stored at 4°C for 4 weeks to prevent volatilization.

Product Description

Free fatty acids (FFA) are non-esterified fatty acids. FFA in serum is mainly an intermediate product of fat metabolism. It is an important metabolic substrate for energy metabolism of body cells and provides energy for metabolism of heart, liver, skeletal muscle and other organs. The detection of free fatty acids is of great significance in clinical practice, which can be used to evaluate the metabolic status and disease risk of organisms. This kit is suitable for the determination of animal tissues, plant tissues, edible oil and other samples. AK1041-100T-96S / AK1041-100T-96S free fatty acid (FFA) content detection kit and AK1041-100T-96S/AK1041-100T-96S free fatty acid

(FFA) content detection kit (enzymatic method) were recommended for serum samples. AK1041-100T-96S/AK1041-100T-96S free fatty acid (FFA) content detection kit (enzymatic method) was recommended for cell samples.

The free fatty acid in the sample was dissolved in hydrocarbons, and reacted with the chromogenic solution

of pyridine-copper ion to form a copper soap complex. There was a characteristic absorption peak at 710 nm, according to which the FFA content could be calculated.

Reagents and Equipment Required but Not Provided.

Visible Spectrophotometer/ Microplate reader, low temperature desk centrifuge, vortex mixer, adjustable pipette, micro glass cuvette/96 well plate(non-polystyrene), n-hexane (≥98%, AR), isooctane (≥98%, AR), ice and distilled water.

Operation 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: The proportion of tissue mass(g): the volume(mL) of Extract solution is 1:5-10 (it is recommended to weigh about 0.1 g of tissue and add 1 mL of Extract solution). Add the Extract solution, homogenize in an ice bath. Extract at room temperature with shaking for 2 hours (wrapped with a sealing film to prevent volatilization), centrifuge at 8000rpm for 10 minutes at 4℃. Take the supernatant and place it on ice for testing.

2.Edible oil and other liquids: 200μL of the sample was mixed with 800μL of the Extract solution, shaken at room temperature for 2h (wrapped with a sealing film to prevent volatilization), centrifuged at 8000rpm, 4 °C for 10min, and the supernatant was placed on ice for testing (the ratio of the sample to the extract can be adjusted according to the absorbance value).

II. Determination procedure:

1. Preheat the spectrophotometer for 30 minutes, adjust the wavelength to 710nm and adjust zero with Extract solution.

2. Before use, the Reagent I was balanced to room temperature.

3. Standard preparation: 10μmol/mL palmitic acid was diluted with Extract solution to obtain 5, 4, 3, 2, 1, 0.5, 0.25μmol/mL standard substances for later use.

4. Add the following reagents successively into the EP tube. Please read the precautions carefully before the experiment:

Reagent (μL)

Test Tube (T)

Standard Tube (S)

Blank Tube (B)

Sample

400

-

-

Standard

-

400

-

Extract solution

-
-
400
Reagent I
200
200
200
Fully shaken for 5 min, centrifuged at 3000 rpm for 10 min at room temperature, and 200 μL of supernatant was taken to determine the absorbance A at 710 nm, which was recorded as A, AT, AS, and
AB respectively. Calculate ΔAT =AT -AB, ΔAS =AS -AB, (Blank tube and standard curve only need to measure 1-2 times).
Note:1. We must ensure that the oscillation frequency and time of each tube are consistent.
2. The upper solution is volatile, please measure as soon as possible after centrifugation, if the sample size is too much, it is recommended to measure in batches.
3. The reagent is corrosive, and the reaction must be determined with a non-polystyrene 96-well plate (such as a 96-well quartz plate) at the end of the reaction.
III. Calculation:
1. Standard curve:
The standard curve was established based on the concentration (x, μmol/mL) and absorbance ΔAS (y, ΔAS) of the standard tube. According to the standard curve, the ΔA determination (y, ΔAT) is substituted into the formula to calculate the sample concentration (x, μmol/mL).
2. Calculation of Free Fatty Acids content:
(1) Calculate by the protein concentration:
FFA content (μmol/mg prot) = x×VE÷(VE×Cpr) ×F=x÷Cpr×F
(2) Calculate by Sample fresh mass:
FFA content (μmol/g mass) = x×VE÷W ×F=x÷W×F
(3) Calculate by the volume of liquid:
FFA content (μmol/mL) =x×(VL×VLE) ÷VL×F=5x×F
VE: The volume of the Extract solution added, 1mL;
Cpr: Sample protein concentration, mg/mL;
W: Sample mass, g;
VLE: Liquid Extract solution, 0.8mL
VL: Pretreatment liquid volume, 0.2mL;
F: Dilution factor;
Notes:
1． When the ΔAT>0.5, the sample can be appropriately diluted with Extract solution for measurement. If the ΔAT <0.01, It can increase the sample mass or increase the liquid extraction volume (while reducing the volume of the extract), and pay attention to modify the calculation formula.
2． The Extract solution is an organic solvent, if calculated according to the protein concentration of the

sample, it is necessary to take another tissue to determine the protein concentration.

3．Since the Extract solution is an organic reagent, multiple absorptions of the same pipette will cause inaccurate volume, it is recommended to replace the pipette in time.

Experimental example:

1.

Take 0.1g of Rat liver and add 1mL of Extract solution to the ice bath homogenate, take the supernatant and follow the determination steps. Measured using a 96 well plate and calculated ΔAT=AT-AB=0.155-0.055=0.100. Substituting the standard curve y=0.0702x -0.0143, R2=0.9982, and the FFA content is calculated according to the sample mass:

FFA content (μmol/g mass) =x÷W×F=16.282 μmol/g mass

2.

Take 0.1g of peanut and add 1mL of Extract solution to the ice bath homogenate, take the supernatant was diluted 2 times. and follow the determination steps. Measured using a 96 well plate and calculated ΔAT=AT-AB=0.317-0.055=0.262. Substituting the standard curve y=0.0702x -0.0143, R2=0.9982, and the FFA content is calculated according to the sample mass:

FFA content (μmol/g mass) =x÷W×F=78.718 μmol/g mass

3.

Take some Edible oil, and follow the determination steps. Measured using a 96 well plate and calculated ΔAT =AT -AB =0.429-0.055 =0.374. Substituting the standard curve y=0.0702x -0.0143, R2=0.9982, and the FFA content is calculated according to the volume of liquid:

FFA content (μmol/mL) =5x×F =27.657μmol/mL