Technical Manual

Non-esterified Free Fatty Acids (NEFA) Colorimetric Assay Kit

- **Catalogue Code: MAES0028**
- **Size: 96T**
- **Research Use Only**

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.01-3.0 mmol/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Free fatty acids, also known as non-esterified fatty acids, are derived from dietary or the metabolism of adipose tissue. In adipose tissue, hormone-sensitive lipase (HSL) decomposes triglycerides to produce glycerol and fatty acids. Circulating in the body with free fatty acids combined with plasma albumin, used as an energy source easily absorbed by muscles, brains, and other tissues and organs. NEFA is not only the product of fat hydrolysis, but also the substrate of fat synthesis. The concentration of NEFA is related to lipid metabolism, glucose metabolism and endocrine function.

3. Intended Use

This kit can be used for detection of non-esterified free fatty acids (NEFA) content in serum, plasma, tissue homogenate, cells or cell supernatant samples.

4. Detection Principle

NEFA and can react with coenzyme A and form acetyl-CoA under the catalysis of acetyl-CoA-synthetase (ACS). Acetyl-CoA can produce H_2O_2 when catalyzed by acetyl-CoA-oxidase (ACOD). Then H_2O_2 react with TOOS and 4-amino-antipyrine (4-APP) to generate a colored substrate under the catalysis of peroxidase (POD). The colored substrate has a maximum absorption peak at 546 nm. Measure the OD value at 546 nm and calculate the NEFA content indirectly.

5. Kit Components & Storage

Item	Specification	Storage
Working Solution 1	20 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Working Solution 2	5 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Standard (1.04 mmol/L)	0.2 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Biochemical analyzer (546 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)

6. Sample Preparation

Sample requirements: Samples (serum, plasma) can be stored at 2~8°C for 3 days. It is recommended that the samples should be stored at -20°C or lower temperature condition if can't detect immediately. Tissue homogenate and cell homogenate must be detected in that very day. Don't use plasma sample anticoagulated with heparin.

1. Serum (plasma) sample:
Separate serum or plasma just in time after blood collection and prevent hemolysis. It is recommended to detect the sample immediately. (The concentration of NEFA may increase due to the degradation of lipid.).

2. Cell culture supernatant:
Detect directly. If there is turbidity, centrifuge at 3100 g for 10 min. Take the supernatant and preserve on ice before detection. If not detected on the same day, it can be stored at -80°C for a month.

3. Cell sample:
Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add normal saline (0.9% NaCl) at a ratio of cell number (2×10^6): (μL) =1: 200. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve on ice before detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

4. Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of normal saline (0.9% NaCl) (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:
The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:
Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.01-3.0 mmol/L).

7. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 546 nm

8. Operation Table

Main performance index

Main wavelength	546 nm	**Auxiliary wavelength**	600 nm
Reaction method	End-point method	**Reaction temperature**	37°C
Reaction direction	Up reaction (+)		

	Blank tube	Standard tube	Sample tube
Double-diatilled water (μL)	4		
Standard (μL)		4	
Sample (μL)			4
Working Solution 1 (μL)	200	200	200
Mix fully and incubate at 37°C for 5 min. Measure the OD value (A_1) of each tube at 546 nm.			
Working Solution 2 (μL)	50	50	50
Mix fully and incubate at 37°C for 5 min. Measure the OD value (A_2) of each tube at 546 nm wavelength. $\triangle A=A_2-A_1$.			

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9. Calculations

1. Serum (plasma) and other liquid sample:

NEFA content (*mmol/L*)

$$= \frac{\triangle \text{ Asample} - \triangle \text{ Ablank}}{\triangle \text{ Astandard} - \triangle \text{ Ablank}} \times \text{Concentration of standard } (mmol/L) \times f$$

2. Tissue (cell) sample:

NEFA content (*mmol/gprot*)

$$= \frac{\triangle \text{Asample} - \triangle \text{Ablank}}{\triangle \text{Astandard} - \triangle \text{Ablank}} \times \text{Concentration of standard } (mmol/L) \div \text{Protein concentration of sample } (gprot/L)$$

f: Dilution factor of sample before test

10. Performance Characteristics

Detection Range	0.01-3.0 mmol/L

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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