Technical Manual

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

- Catalogue Code: MAES0026
- Size: 96T
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.15-1.5 mmol/L

Sensitivity:

0.15 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 to measure the non-esterified free fatty acids (NEFA) content in animal tissue sample.

4. Detection Principle

Under the condition of weak acidity, NEFA react with nantokite to form copper soap, which has a specific absorption peak at 715nm. The content of NEFA can be calculated indirectly by measuring the OD value at 715 nm.

5. Kit Components & Storage

Item	Specification	Storage
Extracting Solution	60 mL × 2 vials	2-8°C, 6 months
Palmitic Acid Standard (10 mmol/L)	1 mL × 2 vials	2-8°C, 6 months
Control Solution	12 mL ×1 vial	2-8°C, 6 months
Reaction Solution	20 mL × 1 vial	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (690-730 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

6. Assay Notes:

1. The samples should be fresh collected and detect within 24 hours.

2. The supernatant after centrifugation must be clarified for the pre-treatment of tissue samples. Otherwise take the turbid supernatant to another centrifuge tube and centrifuge again.

3. The reagent has a pungent smell. Operate in the draught cupboard.

7. Reagent Preparation:

Bring all reagents to room temperature before use.

8. Sample Preparation

Sample requirements: The samples should be fresh collected and detect within 24 hours.

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 extracting solution (2-8°C) (mL): the weight of the tissue (g) =12:1, homogenize the sample and oscillate at 4°C for 2 hours to extract the NEFA. Centrifuge the sample at 10000 g for 10 min at 4°C and take the supernatant for detection.

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 (0.15-1.5 mmol/L).

The recommended dilution factor for different samples is as follows (for reference only).

Sample Type:	Dilution Factor:
Rat liver tissue homogenate	1
Rat heart tissue homogenate	1
Rat kidney tissue homogenate	1
Mouse liver tissue homogenate	1

Note: The diluent is extracting solution.

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 715 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

10. Operation Steps

The preparation of standard curve

Dilute 10 mmol/L standard solution with extracting solution to a serial concentration. The recommended dilution gradient is as follows: 0, 0.3, 0.4, 0.6, 0.9, 1, 1.2, 1.5 mmol/L.

The measurement of samples

1. **Standard tube:** Add 0.5 mL of standard with different concentrations and add 0.25 mL of reaction solution.
   Control tube: Take 0.5 mL of the supernatant of sample and add 0.25 mL of control solution.
   Sample tube: Take 0.5 mL of the supernatant of sample and add 0.25 mL of reaction solution.

2. Oscillate for 3 min and stand at room temperature for 3 min.

3. Take 0.3 mL of the upper layer liquid to micro-plate and measure the OD value at 715 nm with Microplate reader.

Operation Table

	Standard tube	Sample tube	Control tube
Standard with different concentrations (mL)	0.5		
Sample (mL)		0.5	0.5
Control solution (mL)			0.25
Reaction solution (mL)	0.25	0.25	
Oscillate for 3 min, stand at room temperature for 3 min. Take 0.3 mL of the upper layer liquid to 96-wells microplate and measure the OD value at 715 nm with microplate reader.			

11. Calculations

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

$$\text{NEFA}(\mu\text{mol/g}) = (\Delta A_{715} - b) \div a \times \frac{V_1}{m} \times f$$

y: The absolute OD value of standard ($OD_{Standard} - OD_{Blank}$)

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

f: Dilution factor of sample before tested.

ΔA_{715}: $OD_{Sample} - OD_{Control}$

m: The fresh weight of tissue sample, 0.1 g.

V_1: The volume of extracting solution added during the pretreatment of tissue sample, 1.2 mL.

12. Performance Characteristics

Detection Range	0.15-1.5 mmol/L
Sensitivity	0.15 mmol/L
Average recovery rate (%)	101
Average inter-assay CV (%)	5.1
Average intra-assay CV (%)	3.3

Analysis

For rat liver tissue, take 0.1 g of rat liver tissue, add 1.2 mL extracting solution, oscillate at 4°C for 2 hours to extract the NEFA, centrifuge at 10000×g for 10 min, dilute the supernatant with extracting solution for 3 times, carry the assay according to the operation table.

The results are as follows:

Standard curve: y = 0.09174 x – 0.0026, the average OD value of the sample tube is 0.108, the average OD value of the control tube is 0.049, and the calculation result is:

$$\begin{aligned}\textbf{NEFA content (μmol/g)} &= (\mathbf{0.108 - 0.049 + 0.0026}) \div \mathbf{0.09174} \times \frac{\mathbf{12}}{\mathbf{0.1}} \times \mathbf{3} \\ &= \mathbf{24.17}\end{aligned}$$

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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