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

Catalog Number EEA017 (96 tests)
Rev 3.0

For safety and biohazard guidelines, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

This kit can be used to measure the non-esterified free fatty acids (NEFA) content in animal tissue samples. 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.

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.

Contents and storage

Kit and components are shipped at 2-8℃. An unopened kit can be stored at 2-8℃ for 12 months.

Components	Quantity (96 tests)
Extracting Solution	60 mL × 2 vials
10 mmol/L Palmitic Acid Standard	1 mL × 2 vials
Control Solution	12 mL
Reaction Solution	20 mL
Microplate	1 plate
Plate Sealer	2 pieces

Required materials

- Distilled or deionized water
- Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)
- Microtiter plate reader with software capable of measurement at or near 715 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution
- Multichannel transferpettor (300 μL).

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Sample preparation guidelines

Sample requirements

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

Tissue sample:

- Take 0.1-1 g fresh tissue to wash with homogenization medium at 2-8℃ to remove blood cells.
- Absorb the water with filter paper and weigh.
- Homogenize at the ratio of the extracting solution (mL): the weight of the tissue (g) =12:1, then oscillate at 4℃ for 2 hours to extract the NEFA. Centrifuge the tissue homogenate for 10 min at 10000 g at 4℃.

Prepare samples

It is recommended to take 2~3 samples with expected large difference to do a pre-experiment before formal experiment and 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):

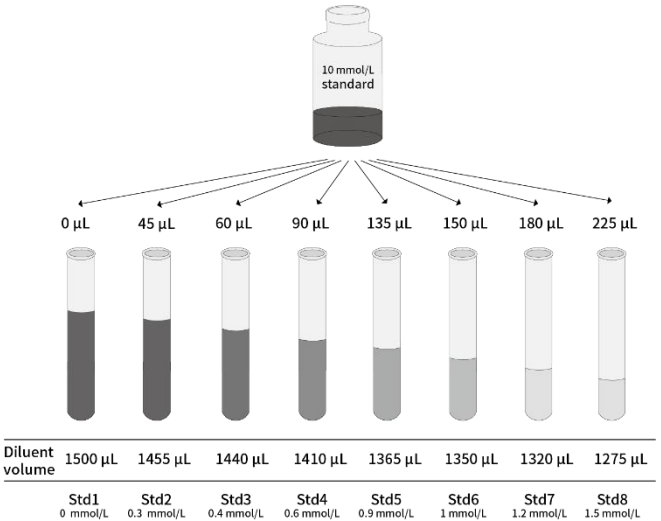
Sample type	Dilution factor
Rat liver tissue homogenate	1
Rat heart tissue homogenate	1
Rat kidney tissue homogenate	1
Mouse liver tissue homogenate	1

Note: The diluent is extracting solution.

Prepare diluted standards

Note: Use glass or plastic tubes for diluting standards.

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.



Assay Procedure

a. Standard tube: add 0.5 mL of standard with different concentrations and add 0.25 mL of reaction solution.

b. Sample tube: take 0.5 mL of the supernatant of sample and add 0.25 mL of reaction solution.

c. Control tube: take 0.5 mL of the supernatant of sample and add 0.25 mL of control solution.

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

e. Take 0.3 mL of the upper layer liquid to microplate.

f. Measure the OD value of each well with microplate reader at 715 nm.

Calculation

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Std1) from all standard readings. This is the absoluted OD value.
3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

Tissue sample:

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

[Note]

ΔA_{715}: Absolute OD value of sample, $OD_{sample} - OD_{control}$;

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;

f: Dilution factor of sample before test.

To easy calculate the test results, refer to the calculation file available on the webpage.

Example analysis

For Rat liver tissue, take 0.1 g of rat liver tissue, add 1.2 mL extracting solution, oscillate at 4℃ for 2 hours to extract the NEFA, centrifuge at 10000×g for 10 min, dilute the supernatant with extracting solution for 3 times, then carry the assay according to the operation table. The results are as follows:

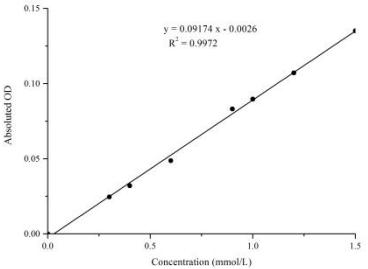
standard curve: y = 0.09174 x – 0.0026, the average OD value of the sample tube is 0.108, the average OD value of the control tube is 0.049, and the calculation result is:

$$\frac{\text{NEFA content}}{(\mu\text{mol/g})} = (0.108 - 0.049 + 0.0026) \div 0.09174 \times \frac{1.2}{0.1} \times 3 = 24.17\ \mu\text{mol/g}$$

Performance characteristics

- Standard curve (example)

The following data were obtained for the various standards over the range of 0–1.50 mmol/L standard.

Concentration(mmol/L)	OD at 715 nm	Standard Curve
1.50	0.178	
1.20	0.150	
1.00	0.132	
0.90	0.126	
0.60	0.091	
0.40	0.075	
0.30	0.067	
0.00	0.043	

- Inter-assay Precision

Three rat liver tissue samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.50	1.00	1.40
%CV	5.5	4.9	4.9

CV = Coefficient of Variation

- Intra-assay Precision

Three rat liver tissue samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.50	1.00	1.40
%CV	3.5	3.4	3.0

CV = Coefficient of Variation

- Expected values

This assay was tested with tissue samples

Sample Type	Range (**mmol/L**)	Average (**mmol/L**)
Rat liver tissue	0.9-1.6	1.55
Mouse liver tissue	1.4-1.9	1.74

- Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 99%.

	Sample 1 (low conc.)	Sample 2 (middle conc.)	Sample 3 (high conc.)
Expected Conc.（mmol/L）	0.5	1	1.4
Observed Conc.（mmol/L）	0.49	1.00	1.39
Recovery rate (%)	98	100	99

- Recommended Plate Set Up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'
[Note]: A-H, standard wells; S1-S40, sample wells; S1'-S40', control wells												

- Sensitivity

The analytical sensitivity of the assay is 0.15 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

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Corporate entity: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

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