TECHNICAL MANUAL

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

- **SKU CODE:** MAES0028
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add working solution 1
- Incubate and measure A1
- Add working solution 2
- Incubate and measure A2
- Calculate results

2. 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.

3. Detection principle

NEFA can react with coenzyme A and form acetyl-CoA under the catalysis of acetyl-CoA-synthetase (ACS). Acetyl-CoA can produce H2O2 when catalyzed by acetyl-CoA-oxidase (ACOD). Then H2O2 reacts 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. The OD value at 546 nm is measured to calculate the NEFA content indirectly.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Working Solution 1	20 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 2	Working Solution 2	5 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 3	1.04 mmol/L Standard	0.2 mL × 1 vial	2-8°C, 12 months, shading light
	Microplate	96 wells	No requirement

Item	Component	Size (96 T)	Storage
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

Biochemical analyzer (546 nm), Micropipettor, Water bath, Incubator, Vortex mixer, Centrifuge

Reagents:

Normal saline (0.9% NaCl), Double distilled water

6. Reagent preparation

Equilibrate all reagents to room temperature before use.

7. Sample preparation

Sample requirements

Samples (serum, plasma) can be stored at 2-8°C for 3 days. It is recommended that samples should be stored at -20°C or lower temperature if they cannot be detected immediately. Tissue homogenate and cell homogenate must be detected the same day. Do not use plasma samples anticoagulated with heparin.

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

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

④ Centrifuge at 10,000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (MAES0177, MAES0402, MAES0401).

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 3×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 3×10^6 cells in 150 µL normal saline (0.9% NaCl) with an ultrasonic cell disruptor at 4°C.

④ Centrifuge at 10,000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (MAES0177, MAES0402, MAES0401).

Cell culture supernatant: Detect directly.

8. The key points of the assay

1. Hemolytic samples will affect the results.

2. If the sample content is beyond the linear range, dilute the sample with normal saline before detection, and multiply by the dilution factor when calculating.

3. Choose the nearest wavelength if the instrument cannot be set to the wavelength required by this kit.

4. Personal protection measures are recommended during operation and the instructions must be strictly followed. The waste liquid must be treated according to environmental protection requirements.

5. The degradation of lipids will lead to increased results if the sample has not been detected as soon as possible.

6. NEFA in serum has individual differences and may increase after eating.

9. 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 (+)

Operation procedure:

Blank well: Double-distilled water 4 μL

Standard well: Standard 4 μL

Sample well: Sample 4 μL

Add Working Solution 1 (200 μL) to all wells.

Mix fully and incubate at 37°C for 5 min. Measure the OD value (A_1) of each tube at 546 nm.

Add Working Solution 2 (50 μL) to all wells.

Mix fully and incubate at 37°C for 5 min. Measure the OD value (A_2) of each tube at 546 nm wavelength. $\Delta A = A_2 - A_1$.

10. Calculation

Serum (plasma) sample and other liquid samples:

NEFA content (mmol/L) = (ΔA_sample - ΔA_blank) / (ΔA_standard - ΔA_blank) × c × f

2. Tissue and cell sample:

NEFA content (mmol/gprot) = (ΔA_sample - ΔA_blank) / (ΔA_standard - ΔA_blank) × c × f ÷ C_{pr}

[Note]

ΔA_blank: The change OD value of blank ($A_2 - A_1$).

ΔA_standard: The change OD value of standard ($A_2 - A_1$).

ΔA_sample: The change OD value of sample ($A_2 - A_1$).

c: Concentration of standard, 1.04 mmol/L.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, gprot/L.

11. Performance index

1. The absorbance of blank tube: A_546nm < 1.000 (optical path = 1.0 cm).

2. Linear range: 0.01-3.0 mmol/L, $r^2 \geq 0.990$.

3. Sensitivity: The ΔA value is more than 0.050 when testing 1.0 mmol/L samples.

4. Accuracy: Relative deviation ≤ 15.0%. Absolute deviation ≤ 0.5 mmol/L.

5. Precision: The intra-assay CV ≤ 10% and the inter-assay CV ≤ 8%.

12. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.