TECHNICAL MANUAL

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

- **SKU CODE:** MAES0027
- **SIZE:** 100 Assays
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Measure optical density at 715 nm

2. Intended use

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

3. Detection principle

Under weakly acidic conditions, NEFA react with nantokite to form copper soap, which has a specific absorption peak at 715 nm. The content of NEFA can be calculated by measuring the optical density value at 715 nm.

4. Kit components & storage

Item	Component	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Extracting Solution	60 mL × 2 vials	60 mL × 3 vials	2-8 °C, 12 months
Reagent 2	10 mmol/L Palmitic Acid Standard	1.8 mL × 1 vial	1.8 mL × 2 vials	2-8 °C, 12 months
Reagent 3	Control Solution	14 mL × 1 vial	28 mL × 1 vial	2-8 °C, 12 months
Reagent 4	Reaction Solution	22.5 mL × 1 vial	45 mL × 1 vial	2-8 °C, 12 months

5. Materials prepared by users

Instruments:

Spectrophotometer (715 nm), Vortex mixer, Micropipettor

Note: The reagents must be stored strictly according to the preservation conditions specified. Reagents from different kits cannot be mixed with each other. For small volume reagents, please centrifuge before use to ensure sufficient reagent recovery.

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 10 mmol/L standard solution with extracting solution to create a serial concentration series. The recommended dilution gradient is: 0.00, 0.10, 0.25, 0.50, 1.00, 1.50, 2.00 mmol/L.

7. Sample preparation

1. **Tissue sample preparation:** 1) Harvest the amount of tissue needed for each assay (initial recommendation 200 mg). 2) Wash tissue in cold PBS (0.01 M, pH 7.4). 3) Homogenize 0.2 g tissue in 2.4 mL extracting solution with a Dounce homogenizer at 4 °C. 4) Oscillate at 4 °C for 2 hours to extract the NEFA. 5) Centrifuge at 10,000 × g for 10 min at 4 °C to remove insoluble material. Collect supernatant and keep it on ice for detection.
2. **Sample dilution:** The recommended dilution factors for different samples are as follows (for reference only): Rat liver tissue homogenate: 1-3, Rat heart tissue homogenate: 1, Rat kidney tissue homogenate: 1, Mouse liver tissue homogenate: 1-3. Note: The diluent is extracting solution. For other sample types, perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. Samples should be freshly collected and analyzed within 24 hours.
2. The supernatant after centrifugation must be clear for tissue sample pretreatment. If the supernatant remains turbid, transfer it to another centrifuge tube and centrifuge again.
3. The reagent has a pungent smell. Please operate in a fume hood.

9. Operating steps

1. Standard tube: Add 1 mL of standards with different concentrations into standard tubes, and add 0.5 mL reaction solution. Control tube: Add 1 mL of sample

supernatant and 0.5 mL control solution into control tubes. Sample tube: Add 1 mL of sample supernatant and 0.5 mL reaction solution into sample tubes.

2. Oscillate for 5 min and stand at room temperature for 5 min.

3. Set spectrophotometer to zero with extracting solution and transfer 0.8 mL of the upper layer liquid into 1 mL cuvette. Measure the optical density value at 715 nm.

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This is the absolute OD value.

3. Plot the standard curve using absolute OD value of standard and corresponding concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or Excel).

The sample:

Tissue sample:

NEFA (μmol/g) = (ΔA_715 - b) ÷ a × V / (m × f)

[Note]

ΔA_715: Absolute OD value, OD_{Sample} - $OD_{Control}$.

V: The volume of extracting solution added during tissue sample pretreatment, 2.4 mL.

m: Fresh weight of tissue, 0.2 g.

f: Dilution factor of sample before test.

11. Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three tissue samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Inter-assay Precision

Three tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

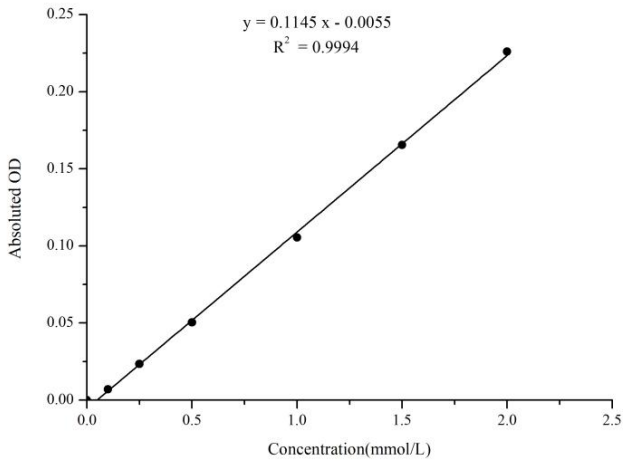
Recovery

Three samples of high, medium, and low concentrations were tested with each concentration analyzed 6 times in parallel to achieve an average recovery rate of 100%.

Sensitivity

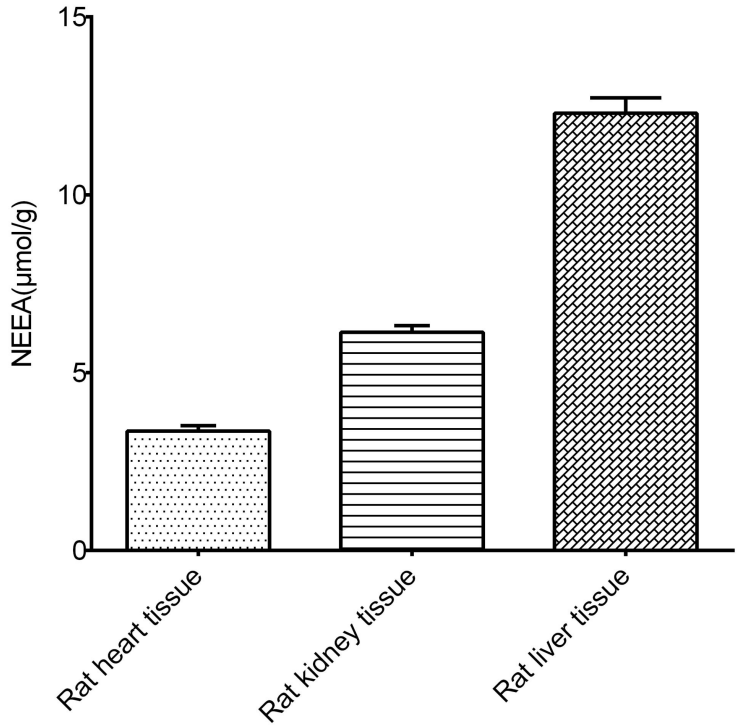
The analytical sensitivity of the assay is 0.05 mmol/L NEFA. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve data



As the OD value of the standard curve may vary according to actual assay performance conditions (e.g., operator, pipetting technique, or temperature effects), the standard curve and data are provided below for reference only:

12. Appendix II Example Analysis



Example analysis:

For rat liver tissue, take 0.2 g of fresh rat liver sample, add 2.4 mL of extracting solution, then homogenize the sample and oscillate at 4 °C for 3 hours to extract the NEFA. Centrifuge the sample at 2,000 × g for 10 min at 4 °C, collect the supernatant, and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 0.10882x - 0.001, the average OD value of the sample tube is 0.109, the average OD value of the control tube is 0.000, and the calculation result is:

NEFA content (μmol/g) = (0.109 + 0.001) ÷ 0.10882 × 12 = 12.13 μmol/g

Detect rat heart tissue (m = 0.2 g, V = 2.4 mL), rat kidney tissue (m = 0.2 g, V = 2.4 mL), rat liver tissue (m = 0.2 g, V = 2.4 mL) according to the protocol.

13. 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.