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Elabscience®

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（**FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !**）

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

Catalog No: E-BC-K013-S
Method: Colorimetric method
Specification: 100 assays **(**Can detect 50 samples without duplication)
Measuring instrument: Spectrophotometer
Sensitivity: 0.05 mmol/L
Detection range: 0.05-2.0 mmol/L

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) Fax: 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Application

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

Detection significance

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.

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 by measuring the OD value at 715 nm.

Kit components

	Components	Specifications	Storage
Reagent 1	Extracting Solution	60 mL × 3 vials	2-8℃, 6 months
Reagent 2	10 mmol/L Palmitic Acid Standard	1.8 mL × 2 vials	2-8℃, 6 months
Reagent 3	Control Solution	28 mL ×1 vial	2-8℃, 6 months
Reagent 4	Reaction Solution	45 mL × 1 vial	2-8℃, 6 months

Experimental instrument

Micropipette, Vortex mixer, Magnetic stirrer, Centrifuge, Spectrophotometry (715 nm), Electronic analytical balance

Pretreatment of sample

[Note]

a) It is recommended to take 2~3 samples which expected large difference to do pre-experiment before formal experiment. Bring all the reagents to room temperature before experiment.

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

1. For blood sample:

Collect the blood sample and stand at room temperature for 1 hour, centrifuge at 2000 g for 15 min at 4℃., then take 0.2 mL of the supernatant. It is recommended to add 2.4 mL of Reagent 1, then oscillate at 4℃ for 3 hours to extract the NEFA. Centrifuge the sample at 2000 g for 10 min at 4℃. and take the supernatant for detection.

2. For tissue sample:

Wash the tissue with PBS (2~8℃, 0.01M, pH=7.4), then remove the water on surface of the tissue with absorbent paper. Weigh and mince the tissue, then add Reagent 1 according to the ratio of Weight (g): Reagent 1(mL) =1: 12, then oscillate at 4℃ for 3 hours to extract the NEFA. Centrifuge the sample at 2000 g for 10 min at 4℃ and take the supernatant for detection.

3. For cell samples:

Add Reagent 1 according to the ratio of cell number (10^6): Reagent 1 (mL)= 5: 1.2 (it is recommende to take 5×10^6 cells), then treat the sample with ultrasonic for 3 min on ice (200 W, 2 seconds/time, interval for 3 seconds), then oscillate at 4℃ for 3 hours to extract the NEFA. Centrifuge the sample at 2000 g for 10 min at 4℃, and take the supernatant for detection.

Operation procedure

1. Dilution of standard

Dilute 10 mmol/L standard solution with reagent 1 to a serial concentration. The recommended dilution gradient is as follows: 2.0, 1.5, 1.0, 0.5, 0.25, 0.1, 0 mmol/L.

2. Operation steps

1) **Standard tube:** Add 1 mL of standard with different concentrations and add 0.5 mL of **Reagent 4**.
   Control tube: Take 1 mL of the supernatant of sample and add 0.5 mL of **Reagent 3**.
   Sample tube: Take 1 mL of the supernatant of sample and add 0.5 mL of **Reagent 4**.
2) Oscillate for 5 min and stand at room temperature for 5 min.
3) Set spectrophotometry to zero with reagent 1 and take 0.8 mL of the upper layer liquid into 1 cm cuvette and measure the OD value at 715 nm.

Note: It can be refer to the following operating table

	Standard tube	Sample tube	Control tube
Standard with different concentrations (mL)	1.0		
Sample (mL)		1.0	1.0
Reagent 3 (mL)			0.5
Reagent 4 (mL)	0.5	0.5	
Oscillate for 5 min, stand at room temperature for 5 min. Set spectrophotometry to zero with reagent 1 and take 0.8 mL of the upper layer liquid into 1 cm cuvette and measure the OD value at 715 nm.			

Calculation of results

Plot the standard curve by using absolute 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 absolute OD value of sample. The standard curve is: y = ax+b.

1. **Calculation formula for blood sample:**

   $$\text{NEFA content } (mmol/L) = (\Delta A_{715}\text{-b}) \div a \times \frac{V1}{V2} \times f$$

2. **Calculation formula for tissue sample:**

   $$\text{NEFA content } (\mu mol/g) = (\Delta A_{715}\text{-b}) \div a \times \frac{V3}{m} \times f$$

3. **Calculation formula for cells sample:**

   $$\text{NEFA content } (\mu mol/10^6 \text{ cell}) = (\Delta A_{715}\text{-b}) \div a \times \frac{V4}{5} \times f$$

   [Note]

   a: the slope of the standard curve.

   b: the intercept of standard curve.

   y: absolute OD value (absolute OD value = the OD value of standard- the OD value of blank)

   x: the concentration of standards.

   V1: the volume of Reagent 1 added during the pretreatment of serum sample, 2.4 mL.

   V2: the volume of serum, 0.2 mL.

   V3: the volume of Reagent 1 added during the pretreatment of tissue sample, 2.4 mL.

   V4: the volume of Reagent 1 added during the pretreatment of cell samples, 1.2 mL.

   m: fresh weight of tissue, 0.2 g.

   5: the cell number of cells is 5×10^6

   f: the dilution multiple of tested samples.

Technical parameters

1. The sensitivity of the kit is 0.05 mmol/L.
2. The intra-assay CV is 2.2 % and the inter-assay CV is 6.1 %.
3. The recovery of the kit is 100 %.
4. The linear range of the kit is 0.05-2 mmol/L.

Notes

1. The kit is for scientific research only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.
5. The samples should be fresh collected and detect within 24 hours.
6. The reagent has a pungent smell. Please operate in the draught cupboard.

Appendix: Standard Curve

(This is for reference only)