PRODUCT INFORMATION & MANUAL

Free Fatty Acids (FFA) Assay Kit (Colorimetric)

NBP3-25927

For research use only.
Not for diagnostic or therapeutic procedures.

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Free Fatty Acids (FFA) Assay Kit (Colorimetric)

Catalog No: NBP3-25927
Method: Colorimetric method
Specification: 96T **(**Can detect 92 samples without duplication)
Measuring instrument: Microplate reader, Biochemistry analyzer
Detection range: 0.01-3.0 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.

Application

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.

Detection principle

NEFA and can react with coenzyme A and form acetyl-CoA under the catalysis of acetyl-CoA-synthetase (ACS). Acetyl-CoA can produce H_2O_2 when catalyzed by acetyl-CoA-oxidase (ACOD). Then H_2O_2 react 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. Measure the OD value at 546 nm and calculate the NEFA content indirectly.

Kit components

Reagent	Component	Concentration	Size	Storage
Reagent 1 (Working Solution 1)	Adenosine triphosphate	5 mmol/L	20 mL ×1 vial	2-8℃ (shading light)
	$MgCl_2$	5 mmol/L		
	Coenzyme A	25 g/L		
	4-amino-antipyrine	5 mmol/L		
	Acetyl-CoA-synthetase	500 U/L		
	Trihydroxy methyl aminomethane buffer	0.1 mol/L		
Reagent 2 (Working Solution 2)	TOOS	10 mmol/L	5 mL ×1 vial	2-8℃ (shading light)
	Acetyl-CoA-oxidase	10 KU/L		
	POD	85 KU/L		
	Trihydroxy methyl aminomethane buffer	0.1 mol/L		
Reagent 3	Standard	1.04 mmol/L	0.2 mL ×1 vial	2-8℃ (shading light)

Experimental instrument

Semi-automatic biochemical analyzer (546 nm), automatic biochemical analyzer (546 nm), Microplate reader (546 nm).

Storage and valid period

This kit can be stored at 2~8℃ in the dark for 6 months. Prevent the reagents from freezing.
Please store the opened reagents can be stored for 1 month at 2~8℃ in the dark.

Sample treatment

1. **Serum or plasma:**
   Separate serum or plasma just in time after blood collection and avoid of hemolysis. It is recommended to detect the sample immediately. (The concentration of NEFA may increase due to the degradation of lipid.)
2. **Tissue sample:**
   Mince the tissues to small pieces, then weighed and homogenized in normal saline on ice, the volume of normal saline (mL): the weight of the tissue (g) =9:1. The tissue homogenate is centrifuged at 2500 rpm for 10 min and take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant.
3. **Cell sample:**
   Collect cells and treat the sample with mechanical homogenate or sonication on ice. Prepared cell homogenate does not require centrifugation. Meanwhile, determine the protein concentration of supernatant.
4. **Cell culture supernatant:**
   Detect directly.

[Note]: Samples (serum, plasma) can be stored at 2~8℃ for 3 days. It is recommended that the samples should be stored at -20℃ or lower temperature condition if can't detect immediately. Tissue homogenate and cell homogenate must be detected in that very day. Don't use plasma sample anticoagulated with heparin.

Operation steps

1. **Main performance index**

Main wavelength	546 nm	Auxiliary wavelength	600 nm
Reaction method	End-point method	Reaction temperature	37℃
Reaction direction	Up reaction (+)		

2. **Operation procedure**

	Blank tube	Standard tube	Sample tube
Double-diatilled water (μL)	4		
Standard (μL)		4	
Sample (μL)			4
Reagent 1 (μL)	200	200	200
Mix fully and incubate at 37℃ for 5 min. Measure the OD value (A1) of each tube at 546 nm.			
Reagent 2 (μL)	50	50	50
Mix fully and incubate at 37℃ for 5 min. Measure the OD value (A2) of each tube at 546 nm wavelength. ΔA=A2-A1.			

Calculation of results

1. **For serum (plasma) and other liquid samples:**

   NEFA content (*mmol/L*)

   $$= \frac{\triangle \text{Asample} - \triangle \text{Ablank}}{\triangle \text{Astandard} - \triangle \text{Ablank}} \times \text{Concentration of standard } (mmol/L)$$

2. **For tissue and cell sample:**

   NEFA content (*mmol/gprot*)

   $$= \frac{\triangle \text{Asample} - \triangle \text{Ablank}}{\triangle \text{Astandard} - \triangle \text{Ablank}} \times \text{Concentration of standard } (mmol/L)$$

   $$\div \text{Protein concentration of sample } (gprot/L)$$

Reference value range

Human serum and plasma: 0.1~0.9 mmol/L

This value is for reference only. It is recommended to establish the own reference value range of each lab.

Performance index

1. **The absorbance of blank tube:** $A_{546\ nm} < 1.000$ (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 test 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%.

Notes

1. The kit is for scientific research only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. Do not use components from different batches of kit.
4. Hemolytic sample will affect the result.
5. If the sample content is beyond linear range, please dilute the sample with normal saline before detection, and multiply the dilution multiple when calculating.
6. Choose the nearest wavelength if the instrument cannot be set to the wavelength required by this kit.
7. Personal protection measures are recommended when operating and the instructions must be strictly obeyed. The waste liquid must be treated according to the environmental protection requirement.
8. The degradation of lipid will lead to the increase of result if the sample has not been detected as soon as possible.
9. NEFA in serum has individual difference and may increase after eating.