Non-esterified Free Fatty Acids Assay Kit

**Catalog No:** E-BC-K014  
**Method:** Colorimetric method  
**Specification:** 96T

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

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

Phone: 240-252-7368(USA)   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 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 H₂O₂ when catalyzed by acetyl-CoA-oxidase (ACOD). Then H₂O₂ 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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Specification</th>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1 (working solution 1)</td>
<td>20 mL × 1 vial</td>
<td>Adenosine triphosphate</td>
<td>5 mmol/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MgCl₂</td>
<td>5 mmol/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coenzyme A</td>
<td>25 g/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4-amino-antipyrine</td>
<td>5 mmol/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acetyl-CoA-synthetase</td>
<td>500 U/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trihydroxy methyl aminomethane buffer</td>
<td>0.1 mol/L</td>
</tr>
<tr>
<td>Reagent 2 (working solution 2)</td>
<td>5 mL × 1 vial</td>
<td>TOOS</td>
<td>10 mmol/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acetyl-CoA-oxidase</td>
<td>10 KU/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>POD</td>
<td>85 KU/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trihydroxy methyl aminomethane buffer</td>
<td>0.1 mol/L</td>
</tr>
<tr>
<td>Reagent 3</td>
<td>0.2 mL × 1 vial</td>
<td>Standard</td>
<td>0.1 mol/L</td>
</tr>
</tbody>
</table>

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 2 weeks 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 (E-BC-K318, E-BC-K168, E-BC-K165).

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 (E-BC-K318, E-BC-K168, E-BC-K165).

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

<table>
<thead>
<tr>
<th>Main wavelength</th>
<th>546 nm</th>
<th>Auxiliary wavelength</th>
<th>600 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction method</td>
<td>End-point method</td>
<td>Reaction temperature</td>
<td>37℃</td>
</tr>
<tr>
<td>Reaction direction</td>
<td>Up reaction (+)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. Operation procedure

<table>
<thead>
<tr>
<th></th>
<th>Blank tube</th>
<th>Standard tube</th>
<th>Sample tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>Double-diatilled water (μL)</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.00 mmol/L Standard (μL)</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample (μL)</td>
<td></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Reagent 1 (μL)</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Mix fully and incubate at 37℃ for 5 min. Measure the OD value (A1) of each tube at 546 nm.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reagent 2 (μL)</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Mix fully and incubate at 37℃ for 5 min. Measure the OD value (A2) of each tube at 546 nm wavelength. ΔA=A2-A1.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Calculation of results

1. For serum (plasma) and other liquid samples:
   NEFA content (mmol/L)
   \[
   \frac{\Delta \text{A}_{\text{sample}} - \Delta \text{A}_{\text{blank}}}{\Delta \text{A}_{\text{standard}} - \Delta \text{A}_{\text{blank}}} \times \text{Concentration of standard (mmol/L)}
   \]

2. For tissue and cell sample:
   NEFA content (mmol/gprot)
   \[
   \frac{\Delta \text{A}_{\text{sample}} - \Delta \text{A}_{\text{blank}}}{\Delta \text{A}_{\text{standard}} - \Delta \text{A}_{\text{blank}}} \times \text{Concentration of standard (mmol/L)}
   \]
   \[
   \div \text{Protein concentration of sample (gprot/L)}
   \]

Reference value range
Human serum and plasma: 0.1~0.77 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\text{nm}} \leq 0.1500.
2. Linear range: 0.01~2.0 mmol/L, r^2 > 0.995.
3. Sensitivity: The ΔA value is between 0.0800~0.2000 when test 1.0 mmol/L samples.
4. Accuracy: Relative deviation ≤ 15.0%. Absolute deviation ≤ 0.1 mmol/L.
5. Precision: The intra-assay CV ≤ 10% and the inter-assay CV ≤ 10%.

Notes
1. This product is for scientific research use only, not for clinical diagnosis.
2. Hemolytic sample will affect the result.
3. If the sample content is beyond linear range, please dilute the sample with normal saline before detection, and multiply the dilution multiple when calculating.
4. Choose the nearest wavelength if the instrument cannot be set to the wavelength required by this kit.
5. Do not use components from different batches of kit.
6. Part of the ingredients in this kit are derived from animal and microorganism. 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.
7. The degradation of lipid will lead to the increase of result if the sample has not been detected as soon as possible.
8. NEFA in serum has individual difference and may increase after eating.