Ver.251201

Non-esterified Free Fatty Acid (NEFA) Assay Kit

OBCK014(50Tests/48Samples)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

1. Product Principle

NEFA can combine with copper ion to form fatty acid copper salt which dissolves in chloroform. Fatty acid copper salt content in chloroform appears direct proportion with NEFA content. As result, it is able to calculate NEFA content by measuring copper ion content by copper reagent.

2. Reagent composition & preparation (50T/48S):

Reagent 1: Chloroform, Prepare by yourself.

Reagent 2: Buffer 40mL×1 bottle, can be stored at room temperature for 3 months.

Reagent 3: Copper reagent, Liquid A 30mL×1 bottle, Liquid B 30mL×1 bottle, Liquid C 5mL×1 bottle. Can be stored at 4℃ for 3 months.

Reagent 3 copper reagent preparation: Mix Liquid A, Liquid B and Liquid C at ratio of 10:9:1, consider volume according to you need. Prepared reagent can be stored at 4℃for 2 weeks.

Reagent 4: Chromogenic agent, Powder×2 vials, Diluent 10mL×2 bottles, can be stored at 4℃ for 3 months.

Reagent 4 chromogenic agent preparation: Use 1 bottle (10mL) diluent to dissolve 1 vial powder, prepared reagent can be stored at 4℃ for 2 weeks.

Reagent 5: Palmitic acid standard powder×2 vials, Solvent 50mL×1 bottle, can be stored at 4℃ for 3 months.

1000μmol/L palmitic acid standard preparation: Use diluent to dissolve 1 vial powder, adjust volume to 20mL, mix sufficiently (wash small vial of powder completely by solvent.)

Reagent 6: Double distilled water 40mL×1 bottle, can be stored at room temperature.

3. Operation procedure:

I. **Label glass test tubes** (it is suggested to use glass grinding test tubes with stopper in order to prevent reagent volatilization and induce extraction.)

II. **Operation table:**

	Blank tube	Standard tube	Sample tube
Double distilled water (mL)	**0.2**	**0.2**	**-**
1000μmol/ L palmitic acid (mL)	**-**	**0.2**	**-**
Sample to assay (mL)	**-**	**-**	**0.2**
Reagent 2 buffer (mL)	**0.5**	**0.5**	**0.5**
Reagent 3 copper reagent (mL)	**1.0**	**1.0**	**1.0**
Reagent 1 (mL)	**4.0**	**3.8**	**4.0**
Extract by mixing sufficiently for 2 minutes, centrifugate at 3500rpm for 10 minutes, remove blue liquid of upper layer and protein clot, take 2mL extract solution of underlayer for chromogenic reaction.			
Underlayer extract (mL)	**2.0**	**2.0**	**2.0**
Chromogenic agent (mL)	**0.25**	**0.25**	**0.25**
Mix sufficiently, place at room temperature for 2 minutes, transfer in cuvettes of 1cm light path, measure OD values of all tubes at 440nm (adjust zero by Reagent 1).			

Note:

1. Extract by mixing sufficiently for 2 minutes accurately (use seconds-counter). If you haven't grinding test tubes, then you can use normal test tubes instead but please seal opening by handi-wrap in order to avoid liquid splashes out of tube.
2. After extraction, centrifugate at 3500rpm for 10 minutes. If underlayer liquid appears semi-coagulated and coagulated layer is quite thick or underlayer liquid is less than 2mL, then you can stir underlayer by small glass bar or micropipet tip softly and centrifugate again to demix clearly.
3. Use injector with anesthesia spinal needle to draw upper layer liquid and discard.
4. Use another injectoe with anesthesia spinal needle to transfer 2.3-2.5mL underlayer extract solution in another test tube. If you draw some upper layer liquid or coagulated layer matter, then centrifuge again or it will affect result. If extract solution appears fog-like turbid, then please place it in 37℃ for 1-2 minutes.
5. Transfer 2mL underlayer extract solution in another test tube, add chromogenic agent for chromogenic reaction.
6. After washing by double distilled water, cuvettes should be rinsed by dehydrated alcohol, then add Reagent 1 to adjust zero. If you skip this step, water droplets will mixed in added Reagent 1 (Reagent 1 and water is insoluble to each other.)
7. You should use glass test tubes in all steps above (can not be instead by plastic test tubes. These 7 steps above are very important to achieve this assay.

4. Calculation

I. **Blood serum NEFA assay:**

Formula:

$$\text{Blood serum NEFA content (μmol/L)} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (1000μmol/L)}$$

II. **Tissue NEFA assay:**

Formula:

$$\text{Tissue NEFA content (μmol/gprot)} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (1000μmol/L)} \div \text{Sample protein concentration (gprot/L)}$$

5. Notes

1. You should use glass test tubes and normal spectrophotometer in this assay, plastic test tubes and semi-automatic/automatic biochemical analysator can not be used (organic solvent is harmful for semi-automatic/automatic biochemical analysator)
2. When you take underlayer extract solution, do not let tip contacts with tube surface in order to avoid take copper reagent. Underlayer extract solution must be limpid or result will be higher than true value.
3. Bilirubin can be extracted by Reagent 1 and disturb spectrophotometry, so auriginous blood serum need 1 contrast tube which use n-butanol to instead of chromogenic agent.