ABSTRACT

Summary:

Quantitative determinations of non-esterified fatty acids in plasma/serum/lymph will be made using the NEFA-HR enzymatic colorimetric method assay.
**MATERIALS**

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- HR Series NEFA-HR(2) Color Reagent A  
  FUJIFILM Wako Diagnostic  
  U.S.A. Catalog #999-34691

- HR Series NEFA-HR(2) Solvent A  
  FUJIFILM Wako Diagnostic  
  U.S.A. Catalog #995-34791

- HR Series NEFA-HR(2) Color Reagent B  
  FUJIFILM Wako Diagnostic  
  U.S.A. Catalog #991-34891

- HR Series NEFA-HR(2) Solvent B  
  FUJIFILM Wako Diagnostic  
  U.S.A. Catalog #993-35191

- NEFA Standard Solution  
  FUJIFILM Wako Diagnostic  
  U.S.A. Catalog #276-76491

Reagent Preparation:

**Working Color Reagent Solutions A:**

*Reagents and Materials:*

- Color Reagent A
- Solvent A

*Procedure:*

Reconstitute Color Reagent A with a portion of Solvent A and then transfer entire contents into Solvent A bottle, rinsing Color Reagent vial several times.

**Working Color Reagent Solutions B:**

*Reagents and Materials:*

- Color Reagent B
- Solvent B

*Procedure:*

Reconstitute Color Reagent B with a portion of Solvent B and then transfer entire contents into Solvent B bottle, rinsing Color Reagent vial several times.

**Note:**

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1. Prepare working Color Reagent Solutions A and B.
   A. Reconstitute Color Reagent A with a portion of Solvent A and then transfer entire contents into Solvent A bottle, rinsing Color Reagent vial several times.

   B. Reconstitute Color Reagent B with a portion of Solvent B and then transfer entire contents into Solvent B bottle, rinsing Color Reagent vial several times.
2 Locate working Standard (1mmol/L or 1 mEq/L).

*THIS ASSAY DOES NOT REQUIRE A SERIAL DILUTION*

3 Using a 96 well flat bottom plate, into separate wells, pipette 5μL of deionized water, 1mMstandard, or sample to be assayed.

4 Add 200μL of **Color Reagent Solution A** to all wells.

5 Mix well and Incubate plate for 5 minutes at 37°C.

6 Measure the absorbance of each well at 550nm (sub:660nm). This measurement (Abs1) will serve as the sample blank.

7 Add 100μL of **Color Reagent Solution B** to all wells.

8 Mix well and Incubate plate for 5 minutes at 37°C.

9 Measure the absorbance of each well at 550nm (sub:660nm). This will be your Abs2 value.

10 Obtain the final absorbance (Sample_{abs}) by subtracting the first reading (step 5) from the second reading (step 8). *
Plot the absorbance vs. concentration to construct the calibration curve. A linear calculation model should be used.

To calculate sample concentration by calculation use the following formula:

\[
\text{Sample Conc.} = \frac{\text{Sample Absorbance}}{\text{Standard Absorbance}} \times \text{Standard Concentration}
\]

*The sample blank absorbance (Abs1) from the first measurement (step 5) should be multiplied by a Factor (F) in order to correct for changes in volume, as follows:

\[
F = \frac{\text{Sample vol} + \text{R1 vol}}{\text{Sample vol} + \text{R1 vol} + \text{R2 vol}}
\]

For this assay:  \( F = \frac{5+200}{5+200+100} = 0.67 \)

Therefore:  \( \text{Sample}_{\text{abs}} = \text{Abs2} - (\text{Abs1} \times 0.67) \)

**Specimen:** Serum or Plasma. Specimen stable for 7 days at 2-8°C or 3 months at -20°C.

**Assay Linearity:** 4.0 mEq/L

**Reagent Stability:** 7 days at 2-8°C

**Stability of Final Reaction:** 60 minutes