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We use this protocol and it's working

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U Cinn - NEFA Concentration

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ABSTRACT

Summary:

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

Keywords: non-esterified fatty acids, NEFA-HR enzymatic colorimetric method

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:

FUJIFILM Wako [RRID:SCR_013651](https://www.fujifilm-wako.com/RRID:SCR_013651)

- 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). *

11 Plot the absorbance vs. concentration to construct the calibration curve. A linear calculation model should be used.

12 To calculate sample concentration by calculation use the following formula:

Sample Conc. = (Sample Absorbance/Standard Absorbance) * 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:

$F = (\text{Sample vol} + \text{R1 vol}) / (\text{Sample vol.} + \text{R1 vol} + \text{R2 vol})$

For this assay: **$F = (5+200) / (5+200+100) = 0.67$**

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

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