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Working

# 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.

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=196&docType=Protocol

MATERIALS

| NAME | CATALOG # | VENDOR | CAS NUMBER | RRID |
|---|---|---|---|---|
| HR Series NEFA-HR(2) Color Reagent A | 999-34691 | FUJIFILM Wako Diagnostic U.S.A. | | |
| HR Series NEFA-HR(2) Solvent A | 995-34791 | FUJIFILM Wako Diagnostic U.S.A. | | |
| HR Series NEFA-HR(2) Color Reagent B | 991-34891 | FUJIFILM Wako Diagnostic U.S.A. | | |
| HR Series NEFA-HR(2) Solvent B | 993-35191 | FUJIFILM Wako Diagnostic U.S.A. | | |
| NEFA Standard Solution | 276-76491 | FUJIFILM Wako Diagnostic U.S.A. | | |

MATERIALS TEXT

**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**

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=(Sample vol + R1 vol) / (Sample vol. + R1 vol + R2 vol)**

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

Therefore: $\mathbf{Sample_{abs} = Abs2 - (Abs1 * 0.67)}$

**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