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U Cinn - NEFA Concentration

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We use this protocol and it's working

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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.

Materials

MATERIALS

- HR Series NEFA-HR(2) Color Reagent A **FUJIFILM Wako Pure Chemical Corporation** **Catalog** #999-34691
- HR Series NEFA-HR(2) Solvent A **FUJIFILM Wako Pure Chemical Corporation** **Catalog** #995-34791
- HR Series NEFA-HR(2) Color Reagent B **FUJIFILM Wako Pure Chemical Corporation** **Catalog** #991-34891
- HR Series NEFA-HR(2) Solvent B **FUJIFILM Wako Pure Chemical Corporation** **Catalog** #993-35191
- NEFA Standard Solution **FUJIFILM Wako Pure Chemical Corporation** **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

Troubleshooting

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