NEFA-HR Assay
C1057

Version: 1
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Summary:

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

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
HR Series NEFA-HR(2) Color Reagent A	Wako	999-34691
HR Series NEFA-HR(2) Solvent A	Wako	995-34791
HR Series NEFA-HR(2) Color Reagent B	Wako	991-34891
HR Series NEFA-HR(2) Solvent B	Wako	993-35191
NEFA Standard Solution	Wako	276-76491

Protocol:

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

Reagent Preparation:

Working Color Reagent Solutions A
Working Color Reagent Solutions B

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.