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# Yale - Non-Esterified Fatty Acids

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#### External link: <u>https://mmpc.org/shared/document.aspx?id=214&docType=Protocol</u>

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Protocol status: Working We use this protocol and it's working

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## Abstract

### Summary:

Procedure used to determine the concentration of NEFA in blood, serum, and plasma. NEFA; Free fatty acids are measured in a multistep reaction to form an colored adduct of 3-methyl-Nethyl-N-(b-hydroxy-ethyl)-analine and 4-aminoantipyridine monitored at 560 nm.

## **Materials**

MATERIALS

X NEFA Reagents A & B Wako Catalog #H7587-58

X NEFA Solvents A & B Wako Catalog #H7587-58

**Reagent Preparation:** 

NEFA Reagent A: Add 50 mL of Solvent A to Reagent A. Gently invert and allow 15 minutes to mix.

NEFA Reagent B: Add 25 mL of Solvent B to Reagent B. Gently invert and allow 15 minutes to mix.

NEFA Solvents A & B: As supplied by vendor.

Note:

Wako <u>RRID:SCR\_013651</u>

Before start

Analysis by automated system Cobas Mira Plus

- 1 Calibrate Cobas for NEFA analysis by running a NEFA standard.
- 2 Sample handling as performed by the Cobas Mira Plus
  - a) Pipette 6µL of sample into cuvette.
  - b) Add 225  $\mu$ L of NEFA Reagent A Mixture.
  - c) Add 75  $\mu L$  of NEFA Reagent B Mixture.
  - d) Mixture is incubated at 37°C for 10 minutes
  - e) Absorbance is measured at 560 nm.