



## Non-esterified fatty acids (NEFA)

Version: 1

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**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-N-ethyl-N-(b-hydroxy-ethyl)-aniline and 4-aminoantipyridine monitored at 560 nm.

### Reagents and Materials:

Reagent/Material	Vendor	Stock Number
NEFA Reagents A & B	Wako	H7587-58
NEFA Solvents A & B	Wako	H7587-58

**Protocol:** 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 6 $\mu$ L 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.

### 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.