



May 17,  
2019

Working

## Yale - Non-Esterified Fatty Acids [↗](#)

John Stack<sup>1</sup>, Gary Cline<sup>1</sup>

<sup>1</sup>Yale University

[dx.doi.org/10.17504/protocols.io.y38fyrw](https://doi.org/10.17504/protocols.io.y38fyrw)

Mouse Metabolic Phenotyping Centers  
Tech. support email: [info@mmpc.org](mailto:info@mmpc.org)

Lili Liang

### 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.

### EXTERNAL LINK

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

### MATERIALS

NAME	CATALOG #	VENDOR
NEFA Reagents A & B	H7587-58	<a href="#">Wako</a>
NEFA Solvents A & B	H7587-58	<a href="#">Wako</a>

### MATERIALS TEXT

#### 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 [RRID:SCR\\_013651](#)

### BEFORE STARTING

*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 µL of NEFA Reagent A Mixture.
  - c) Add 75 µL of NEFA Reagent B Mixture.
  - d) Mixture is incubated at 37°C for 10 minutes

e) Absorbance is measured at 560 nm.



This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited