UC Davis MMPC-Live Protocol
Non-Esterified "free" Fatty Acids (NEFA)

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Summary:

The Wako enzymatic method relies upon the acylation of coenzyme A (CoA) by the fatty acids in the presence of added acyl-CoA synthetase (ACS). The acyl-CoA thus produced is oxidized by added acyl- CoA oxidase (ACOD) with generation of hydrogen peroxide, in the presence of peroxidase (POD) permits the oxidative condensation of 3-methy-N-ethyl-N(β-hydroxyethyl)-aniline (MEFA) with 4-aminoantipyrine to form a purple colored adduct which can be measured colorimetrically at 550 nm.

Reagents and Materials:

Reagent/Material	*Vendor*	*Stock Number*
Calibrator	Wako	276-76491
Reagents	Wako	999-34691 995-34791 991-34891 993-35191
Microplate		
Platereader		

Protocol:

1. Reconstitute Color Reagent A with 50 ml of Solvent A and Color Reagent B with Solvent B.
2. Add 5 µl of calibrator and sample to each well.
3. Add 200 µl of Reagent A to each well. Incubate at 37°C for 5 minutes. Read at 560 nm.

> IMPORTANT: Make sure not to add any bubbles to the wells when dispensing reagents, this will interfere with reading in the platereader.

4. Add 100 µl of Reagent B to each well. Incubate at 37°C for 5 minutes. Read at 560 nm.
5. Subtract blank readings from final readings. The assay will be linear so the unknown samples can be calculated as (Sample Absorbance ÷ Calibrator Absorbance) × Calibrator Concentration.

Reagent Preparation:

Reagent A – reconstitute Color Reagent A with Solvent A

Reagent B – reconstitute Color Reagent B with Solvent B