ABSTRACT

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.

MATERIALS

Reagent Preparation:

Reagent A – reconstitute Color Reagent A with Solvent A
Reagent B – reconstitute Color Reagent B with Solvent B

Note:

FUJIFILM Wako RRID:SCR_013651
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 plate reader.

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.