



Free Fatty Acid Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1155

(Version 3.2C)

Detection and Quantification of Free Fatty Acid(FFA) Content in
Serum, Plasma, Urine, Saliva, Milk, Cell cultures and Other biological
fluidsSamples.

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

Fatty acids are aliphatic monocarboxylic acids that are ubiquitously found in animal or vegetable fat, oil and wax. Fatty Acids play important roles in cellular synthesis, energy metabolism and are implicated in diverse disorders such as diabetes mellitus, sudden infant death syndrome and Reye Syndrome.

Free Fatty Acid Colorimetric Microplate Assay Kit is a sensitive assay for determining Free Fatty Acid concentration in various samples. Fatty acids are converted to their CoA derivatives, which are subsequently oxidized by the enzyme mix to yield an intermediate. Formed intermediate then reacts with the Free Fatty acid probe to generate color which can be read by a spectrophotometer at OD 550 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Enzyme	16 mlx 1	4 °C
Dye Reagent	4 mlx 1	4 °C
Standard (1 mmol/L)	200 µlx 1	4 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 550 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Ethanol
6. Centrifuge
7. Timer

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 1/0.5/0.25/0.125/0.0625/0.0312/0.0156 mmol/L.

V. SAMPLE PREPARATION

1. For serum, plasma or other liquid samples

Detect directly.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml ethanol, centrifuged at 10,000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube for detection.

VI. ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent*	Sample**	Standard	Blank
Enzyme	160 μ l	160 μ l	160 μ l
Sample	20 μ l	--	--
Standard	--	20 μ l	--
Distilled water	--	--	20 μ l
Mix, incubate at room temperature for 5 minutes.			
Dye Reagent	40 μ l	40 μ l	40 μ l
Mix, incubate at room temperature for 10 minutes, record absorbance measured at 550 nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**The concentrations can vary over a wide range depending on the different samples.

For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(OD_{\text{Sample}} - OD_{\text{Blank}}) - \text{Intercept}}{\text{Slope}} \times n (\text{mmol/L})$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the weight of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}})} (\text{mmol/g})$$

2.2 According to the volume of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times V_{\text{Sample}}} (\mu\text{mol/ml})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Standard} : the standard concentration, mmol/L = $\mu\text{mol/ml}$

V_{Standard} : the volume of standard in assay procedure, μl

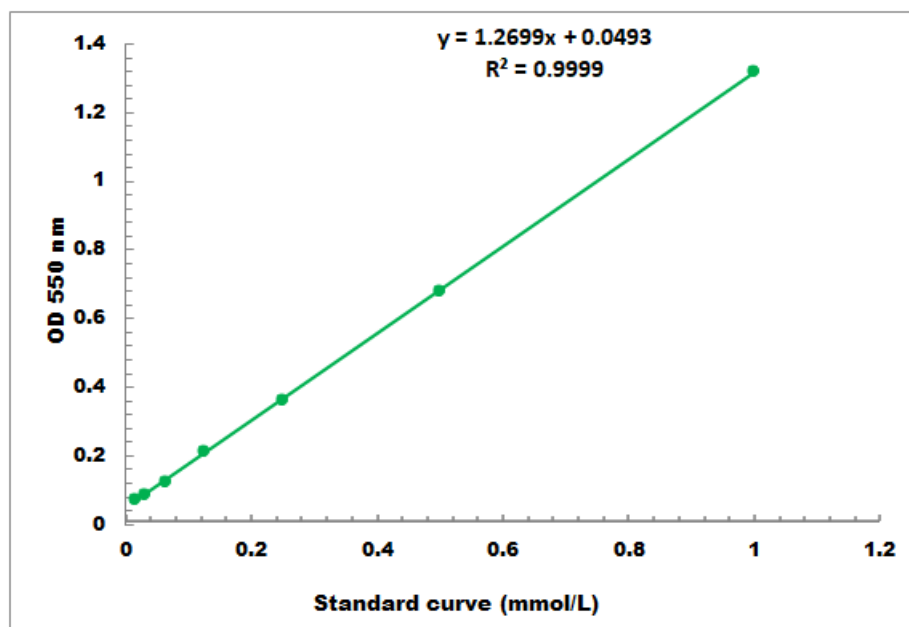
V_{Sample} : the volume of sample in assay procedure, μl

V_{Assay} : the volume of Assay Buffer, μl

W: the weight of sample, g

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.01 mmol/L - 1 mmol/L