Free Fatty Acid

Microplate Assay Kit

User Manual

Catalog # CAK1155

(Version 3.1C)

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

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 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 ml x 1	4 °C
Dye Reagent	4 ml x 1	4 °C
Standard (1 mmol/L)	200 µl x 1	4 °C
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Note:

Standard: dilute with distilled water.

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. 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.

V. ASSAY PROCEDURE

Add following reagents in the microplate:

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

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the liquid of sample

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

$$= (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

1. According to the weight of sample

$$FFA\ (\mu mol/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Ethanol})$$

$$= (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

$C_{Standard}$: the concentration of standard, 1 mmol/L = 1 μmol/ml;

W: the weight of sample, g;

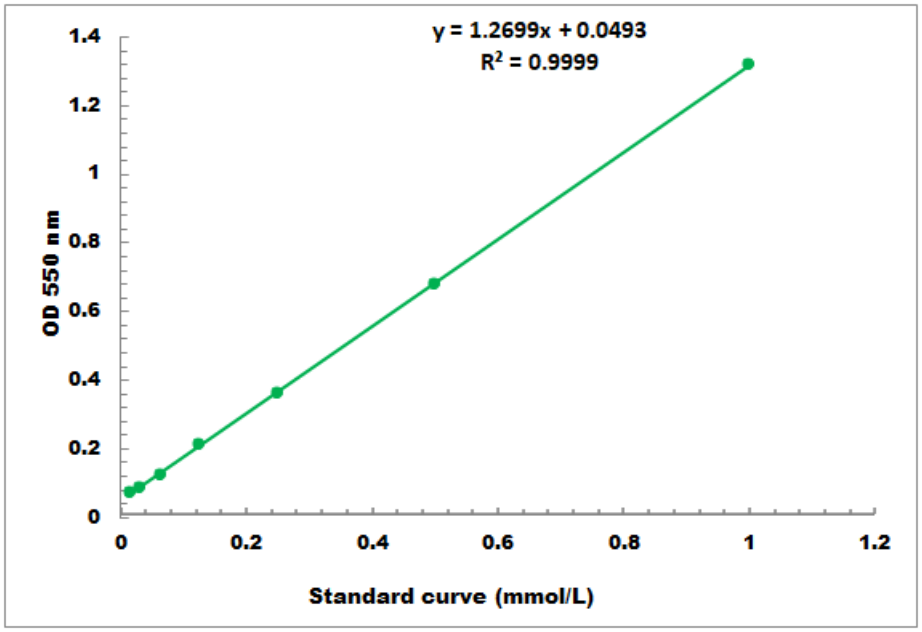
$V_{Standard}$: the volume of standard, 0.02 ml;

V_{Sample}: the volume of sample, 0.02 ml;

$V_{Ethanol}$: the volume of Ethanol, 1 ml.

VII. 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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES