Free Fatty Acid

Microplate Assay Kit

User Manual

Catalog # FTA0141

(Version 1.1A)

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.

I. INTRODUCTION.....2
II. KIT COMPONENTS.....3
III. MATERIALS REQUIRED BUT NOT PROVIDED.....3
IV. SAMPLE PREPARATION.....4
V. ASSAY PROCEDURE.....5
VI. CALCULATION.....6
VII. TYPICAL DATA.....7
VIII. TECHNICAL SUPPORT.....7
IX. NOTES.....7

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. FFA and copper ions combine to fatty acid copper salt. The reaction products can be measured at a colorimetric readout at 550 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Extractant	30 ml x 4	4 °C
Reaction Buffer	Powder x 1	4 °C
Reaction Buffer Diluent	15 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Dye Reagent Diluent	5 ml x 1	4 °C
Standard	Powder x 1	4 °C
Technical Manual	1 Manual	

Note:

Reaction Buffer: add 15 ml Reaction Buffer Diluent to dissolve before use.

Dye Reagent: add 5 ml Dye Reagent Diluent to dissolve before use.

Standard: add 1 ml Extractant to dissolve before use, mix; then add 0.2 ml into 0.8 ml Extractant, the concentration will be 5 mmol/L.

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. Microcentrifuge tube
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 0.5 ml Extractant, 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 microcentrifuge tube:

Reagent	Sample	Standard	Blank
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Extractant	450 µl	450 µl	450 µl
Shake and mix by vortex for 5 minutes, standing for 15 minutes; then shake and mix by vortex for 2 minutes; centrifuged at 4000g for 5 minutes. Absorb the lower liquid into a new microcentrifuge tube.			
Lower liquid	300 µl	300 µl	300 µl
Reaction Buffer	150 µl	150 µl	150 µl
Shake and mix by vortex for 20 minutes; centrifuged at 4000g for 5 minutes. Then add the supernatant into the microplate.			
Supernatant	100 µl	100 µl	100 µl
Dye Reagent	50 µl	50 µl	50 µl
Mix, 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) The Extractant is volatile. It is better to operate it in the fume hood.

VI. CALCULATION

1. According to the liquid of sample

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

$$= 5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

1. According to the weight of sample

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

$$= 2.5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

$C_{Standard}$: the concentration of standard, 5 mmol/L = 5 μmol/ml;

W: the weight of sample, g;

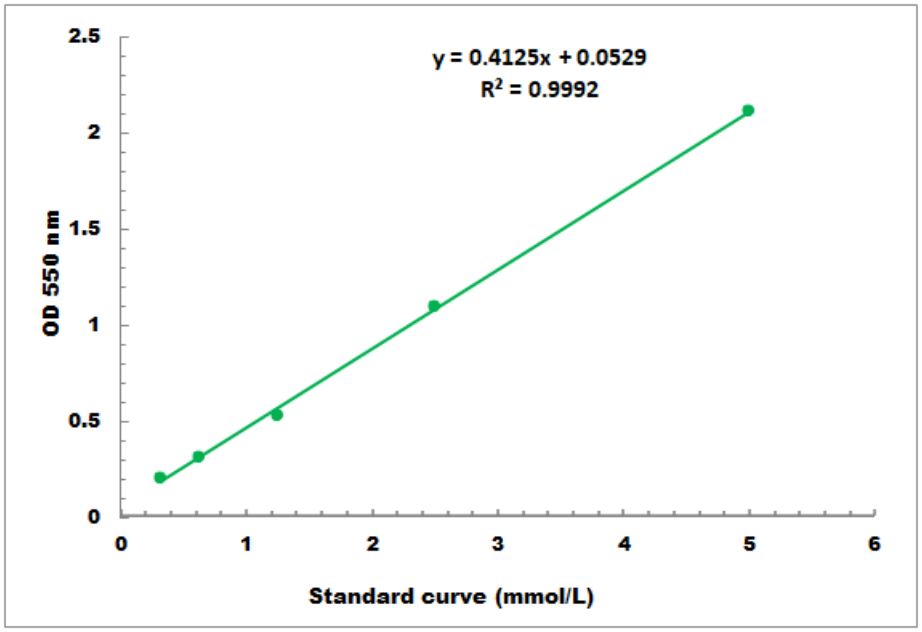
$V_{Standard}$: the volume of standard, 0.01 ml;

V_{Sample}: the volume of sample, 0.01 ml;

$V_{Extractant}$: the volume of Extractant, 0.5 ml.

VII. TYPICAL DATA

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



Detection Range: 0.05 mmol/L - 5 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