96-well Serum/Plasma Fatty Acid Kit
Non-Esterified Fatty Acids Detection
1,000 Point Kit

Cat# SFA-10

INSTRUCTION MANUAL ZBM0029.03

STORAGE CONDITIONS

- **Reagents & Buffers:** 4°C

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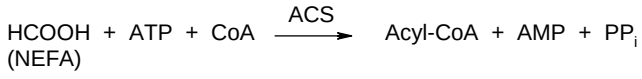
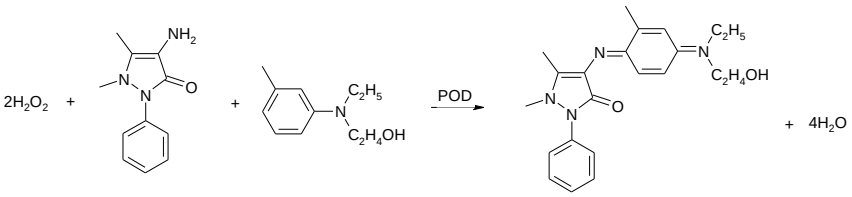
INTRODUCTION

This kit is designed to accurately determine the amount of free fatty acid present in blood serum or plasma of humans, mice, rats, and other animals in a 96-well format for increased throughput analysis. Blood can be collected in plain evacuated tubes or in the presence of common anti-coagulants: sodium citrate, ammonium oxalate and EDTA. **NOTE: Heparin or Heparinized tubes should not be used because this will generate inaccurate readings.** Serum should be separated from clotted blood by centrifugation as soon as possible and may be stored frozen (-20°C) prior to analysis.

PRINCIPLE OF THE ASSAY

Assessment of serum fatty acids is through a coupled reaction to measure non-Esterified fatty acids (NEFA). The initial step, carried out by acyl-CoA synthetase (ACS), produces fatty acyl-CoA thiol esters from the NEFA, ATP, Mg, and CoA in the reaction. The acyl-CoA derivatives react with oxygen in the presence of acyl-CoA oxidase (ACOD) to produce hydrogen peroxide. Hydrogen peroxide in the presence of peroxidase (POD) allows the oxidative condensation of 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline with 4-aminoantipyrine which forms a purple product that absorbs light at 550nm. This allows the concentration of NEFA to be determined from the optical density measured at 540 - 550nm.





ITEMS INCLUDED IN THE KIT

ITEM	DESCRIPTION	Cap Color	UNIT	QTY	STORAGE
Dilution Buffer	100 ml	---	BOTTLE	1	4°C
FFA Standard	1mM Stock. See page 3 for standard curve preparation	AMBER	100 ul/ VIAL	4	4°C
FFA Diluent A		YELLOW	50ML	2	4°C
FFA Diluent B* Warning		PINK	25ML	2	4°C
FFA Reagent A** Warning	Reconstitute each using 50 ml FFA Diluent A. Discard remainder after 10 days	YELLOW	BOTTLE	2	4°C
FFA Reagent B	Reconstitute each using 25 ml FFA Diluent B. Discard remainder after 10 days	PINK	BOTTLE	2	4°C

Other equipment/reagents required but not provided with the kit:

- Multi-channel Pipet , single channel pipet and pipet tips
- Plate reader with a filter of 540 nm
- Incubator at 37ºC
- Large gauge needle
- Tubes for diluting standards
- 96-well clear bottom assay plates
- Disposable trays for dispensing reagents

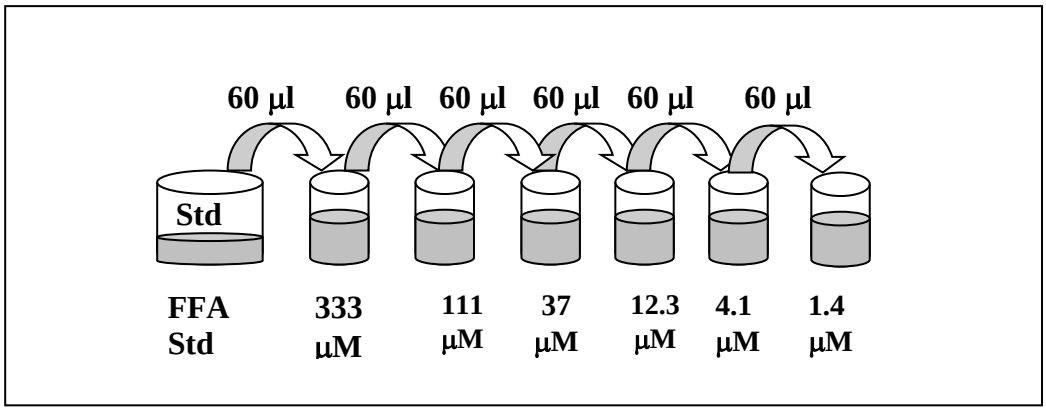
** Warning. May cause an allergic skin reaction. See SDS for more details*

*** Warning. H302 - Harmful if swallowed; H402 - Harmful to aquatic life See SDS for more details*

ASSAY PROCEDURE

1. Prepare the standard curve using the STANDARD SOLUTION as follows:

   Briefly spin down the contents of the free fatty acid standard tube. Standards are: 0, 1.4, 4.1, 12.3, 37, 111, and 333 µM fatty acid. Prepare as follows:The kit standard solution is the 1.0 mM standard. Pipette 120 µl of Dilution Buffer into 6 tubes (not provided). Pipette 60 µl of the FFA Standard Stock into a tube labeled 333 µM. Prepare a dilution series as depicted below. Mix each new dilution thoroughly before proceeding to the next. The Dilution Buffer alone serves as the zero standard.



Note: The above dilution series generates enough volume to perform the standard curve in duplicate. If you wish to perform the standard curve in duplicate, please note that seven fewer data points can be assayed with this kit.

2. Also at this time prepare the FFA Reagent A by adding 50ml FFA Diluent A per bottle and gently invert. DO NOT VORTEX! Store any remaining solution at 2-8°C; it is stable for 10 days after reconstitution refrigerated (2-8°C).

3. Add 5 µl (or 1 - 10 µl) of serum or plasma to a well of 96-well plate (not provided). Add dilution buffer to each well to total 50 µl including serum or plasma sample. **THIS RESULTS IN A 10x DILUTION OF YOUR SAMPLE (5 µl in 50 µl).** Add 50 µl of each standard to empty wells (use another blank plate if necessary).

4. Add the reconstituted FFA Reagent A to a disposable tray (not provided). Add 100 µl of FFA Reagent A to each well. Gently shake the plate to ensure mixing. Place in a 37 °C incubator for 10 minutes.

5. Prepare the FFA Reagent B by adding one 25ml FFA Diluent B per 25ml FFA Reagent B bottle and gently invert. DO NOT VORTEX! Store any remaining solution at 2-8°C; it is stable for 10 days after reconstitution refrigerated (2-8°C).

6. Add the reconstituted FFA Reagent B to another disposable tray (not provided). Add 50 µl of FFA Reagent B to each well. Gently shake the plate to ensure mixing. Place in a 37 °C incubator for

10 minutes. Store any remaining solution at 2-8°C; it is stable for 10 days after reconstitution refrigerated (2-8°C).

7. Allow the plate to equilibrate to room temperature for 5 minutes. During this time, ensure that there are no bubbles in the solution mixture. Use a large gauge needle or clean pipet tip to pop any bubbles as this will result in inaccurate absorbance readings.

8. The optical density of each well is then measured at 540 nm.

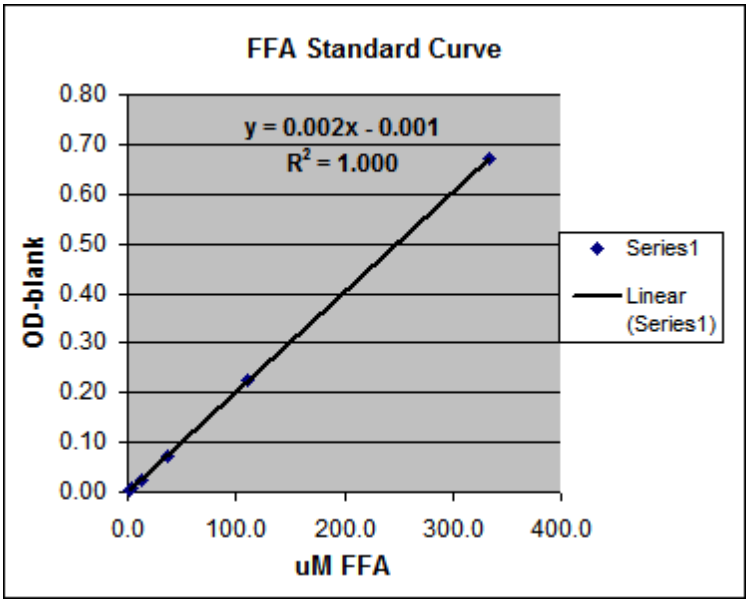
FATTY ACID STANDARD CURVE _____

Generate standard curve: see example below

[DO NOT use this standard curve to generate your data. This is an example.]

Subtract the OD value of the 0μM standard from all OD values including the standard curve. . Note: 1mM standard is commonly omitted from analysis due to lack of linearity between 333 μM and 1mM. Optionally, a 4-parameter fit may be used to calculate standard curve.

uM FFA	OD	OD	OD-blank	OD-blank	Avg OD-blank
0	0.05	0.048			0.049
1.4	0.051	0.053	0.002	0.004	0.003
4.1	0.056	0.058	0.007	0.009	0.008
12.3	0.070	0.075	0.021	0.026	0.024
37	0.119	0.122	0.070	0.073	0.072
111	0.274	0.277	0.225	0.228	0.227
333	0.689	0.750	0.640	0.701	0.671



Slope	0.002
Intercept	-0.001
R^2	1.000

y = observed O.D. minus the blank

x = concentration of FFA in μM

To calculate x for each y, (i.e. to change the observed O.D. into FFA concentration) use the following equation:

y=(slope) times (x) plus intercept

y=mx+b so x=(y-b)/m

x=(y – (-0.001))/0.002 where 0.002= slope of the line and -0.001= y intercept. Be careful to enter the proper sign for the y intercept value as it may be a negative number.

Data are expressed as μM free fatty acids.

REMEMBER TO ACCOUNT FOR THE DILUTION FACTOR IN STEP 3.

OPTION: express data as Fold induction over appropriate vehicle

$$\text{Fold induction} = \frac{\mu\text{M free fatty acids SAMPLE}}{\mu\text{M free fatty acids VEHICLE}}$$

The R^2 value should be equal or greater then 0.98 for the standard curve to be valid. Any R^2 values below 0.98, must have the standard curve run again.

APPENDIX A: SFA-10 PROCEDURE FLOWCHART

ON DAY OF ASSAY

Add 5 µl/well test sample and 45 µl/well Dilution Buffer to a blank 96-well plate.
Add 50 µl/well diluted standard curve to empty wells.

↓

Reconstitute FFA Reagent A using Diluent A.
Add 100µl/well. Incubate 10 minutes @ 37°C.

↓

Reconstitute FFA Reagent B using Diluent B.
Add 50µl/well. Incubate 10 minutes @ 37°C.

↓

Place at room temp. for 5 minutes. Pop any bubbles in each well using a clean pipet tip or large gauge needle.

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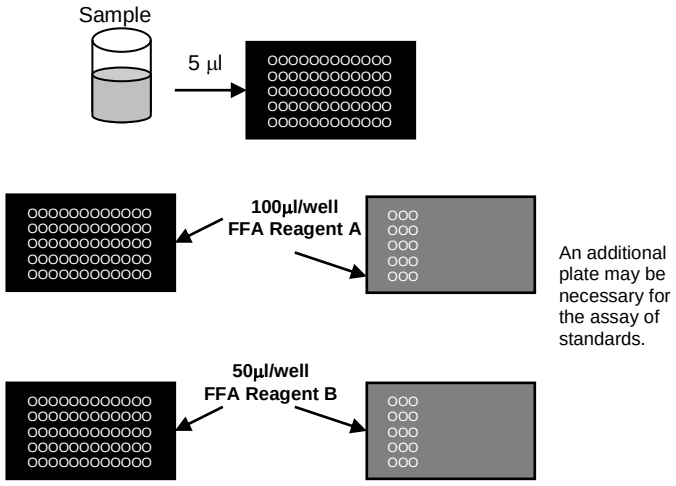
Measure the optical density of each well at 540 nm using a spectrophotometer plate reader.

Reminder: Sample was diluted in Step 3



An additional plate may be necessary for the assay of standards.

APPENDIX B: PLATE LAYOUT _____

H	G	F	E	D	C	B	A	
								1
								2
								3
								4
								5
								6
								7
								8
								9
								10
								11
								12