96-well Serum/Plasma Fatty Acid Kit
Non-Esterified Fatty Acids Detection
100 point kit

Cat# SFA-1

INSTRUCTION MANUAL ZBM0021.02

STORAGE CONDITIONS

- **Reagents & Buffers:** 4°C
- **Blank assay plates (96-well):** Room Temperature

For *in vitro* Use Only

LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, expressed or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Zen-Bio, Inc. Zen-Bio, Inc. shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

ORDERING INFORMATION AND TECHNICAL SERVICES

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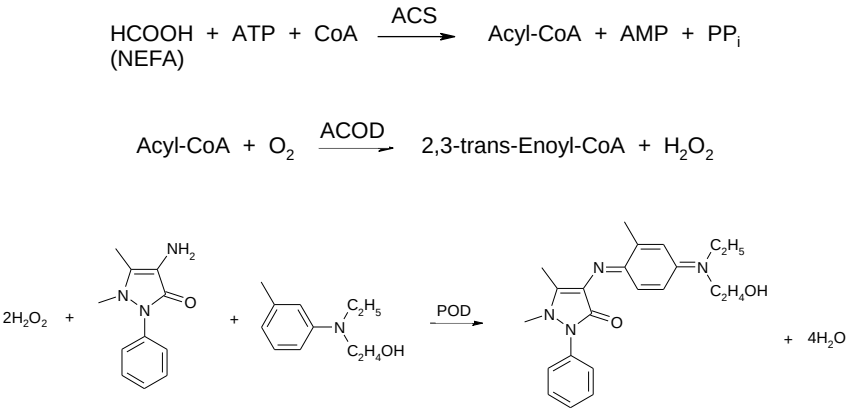
INTRODUCTION

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
Assay Plate, Plate	96-well assay plate, blank	---	PLATE	2	-----
Dilution Buffer	12 ml	---	BOTTLE	1	4°C
FFA Standard	1mM Stock. See page 3 for standard curve preparation	AMBER	100 µl / VIAL	1	4°C
FFA Diluent A		YELLOW LABEL	10.5ML	1	4°C
FFA Diluent B		PINK LABEL	5.5ML	1	4°C
FFA Reagent A	Reconstitute using 10.5 ml FFA Diluent A. Discard remainder after 10 days	YELLOW LABEL	BOTTLE	1	4°C
FFA Reagent B	Reconstitute using 5.5 ml FFA Diluent B. Discard remainder after 10 days	PINK LABEL	BOTTLE	1	4°C
Tray	For multi-channel pipetters, clear polyvinyl	CLEAR	EACH	2	-----

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

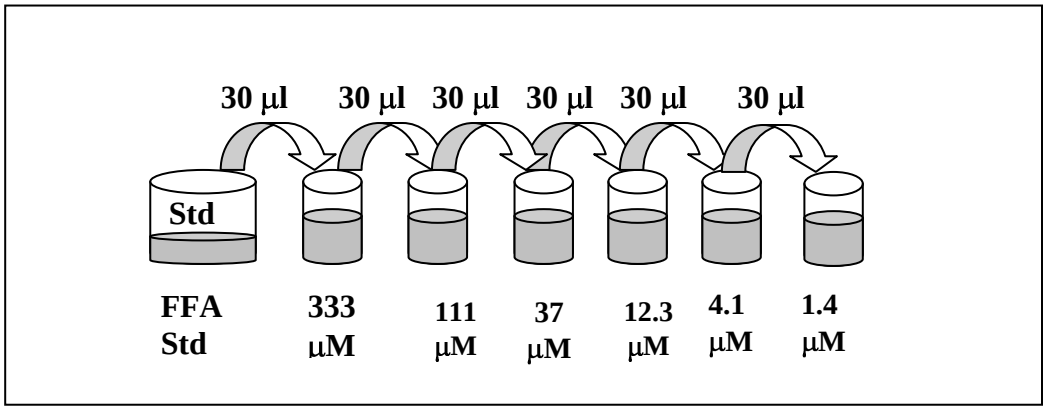
- Multi-channel Pipet , single channel pipet and pipet tips
- Plate reader with a filter of 540 nm
- Incubator at 37°C
- Large gauge needle
- Tubes for dilution of standards

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 before reconstitution. 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 60 μl of Dilution Buffer into 6 tubes (not provided). Pipette 30 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.



2. Also at this time prepare the FFA Reagent A by adding 10.5ml 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 Plate A. 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 PLATE B if necessary).

4. Add 10.5 ml of the reconstituted FFA Reagent A to one of the disposable trays provided in the kit. Add 100 μl of FFA Reagent A to each well. Gently shake the plate to ensure mixing. Place in a 37 ^{o}C incubator for 10 minutes.

5. Prepare the FFA Reagent B by adding 5.5ml FFA Diluent B 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).

6. Add 5.5 ml of the reconstituted FFA Reagent B to the other disposable tray provided in the kit. Add 50 μl of FFA Reagent B to each well. Gently shake the plate to ensure mixing. Place in a 37 ^{o}C incubator for 10 minutes.

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.

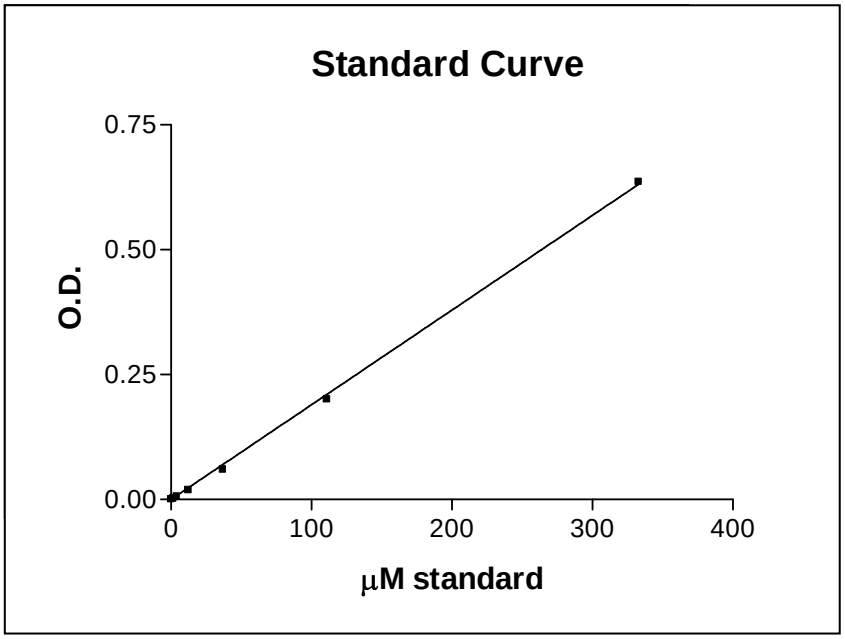
FATTY ACID STANDARD CURVE _____

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.

μM std	OD	OD - zero
333	0.68	0.636
111	0.244	0.2
37	0.104	0.06
12.3	0.063	0.019
4.1	0.05	0.006
1.4	0.046	0.002
0	0.044	0

y = 0.0019x - 0.0045
R^2 = 0.9995



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: PLATE LAYOUT _____

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

APPENDIX B: SFA-1 PROCEDURE FLOWCHART

ON DAY OF ASSAY

Add 5 µl/well test sample and 45 µl/well dilution buffer to one of the blank assay plates provided. 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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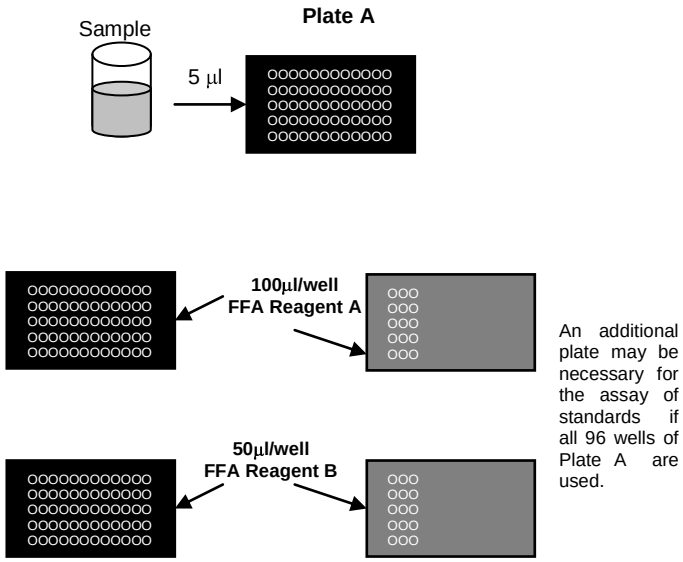
Measure the optical density of each well at 540 nm using a spectrophotometer plate reader.

Reminder: Sample was diluted in Step 3



An additional plate may be necessary for the assay of standards if all 96 wells of Plate A are used.