# Non-esterified fatty acids

Version: 1
Edited by: Jason Kim

*(note that the following list should be linked to the appropriate location.)*



## Summary: *(This area will include a brief description of what the protocol is used for and why someone would need to use it.)*

This experiment measures serum and plasma concentrations of non-esterified fatty acids using a 96-well kit. The experiment involves a coupled reaction to measure non-esterified fatty acids (NEFA) which ultimately 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. Serum fatty acids levels reflect systemic lipid metabolism, lipid digestion/absorption, and lipid clearance. Serum fatty acids levels are altered in obesity, insulin resistance, and type 2 diabetes.

## Reagents and Materials: *(This should be a comprehensive list of stock solutions and material. The reagent list for the stock solutions is included in the reagent preparation area that is included at the end of this SOP.)*

| Reagent/Material | Vendor | Stock Number |
|---|---|---|
| 96-well assay plate, blank | Zen-Bio | SFA-1 |
| Dilution Buffer | Zen-Bio | SFA-1 |
| FFA Standard | Zen-Bio | SFA-1 |
| FFA Diluent A | Zen-Bio | SFA-1 |
| FFA Diluent B | Zen-Bio | SFA-1 |
| FFA Reagent A | Zen-Bio | SFA-1 |
| FFA Reagent B | Zen-Bio | SFA-1 |
| Multichannel Pipette Tray | Zen-Bio | SFA-1 |

| **Additional Items** |
|---|
| • Multi-channel Pipet , single channel pipet and pipet tips |
| • Plate reader with a filter of 540 nm |
| • Incubator at 37oC |
| • Tubes for dilution of standards |

# Protocol:

**Notes:**

- ✓ Freshly prepared blood or plasma samples are recommended. If storing samples, keep blood and plasma samples at –20$^{o}$ C or at -70$^{o}$ C for long-term storage. Avoid freeze/thaw cycles.
- ✓ Avoid using samples with gross hemolysis or lipemia.
- ✓ Allow all reagents to come to room temperature before measurement.

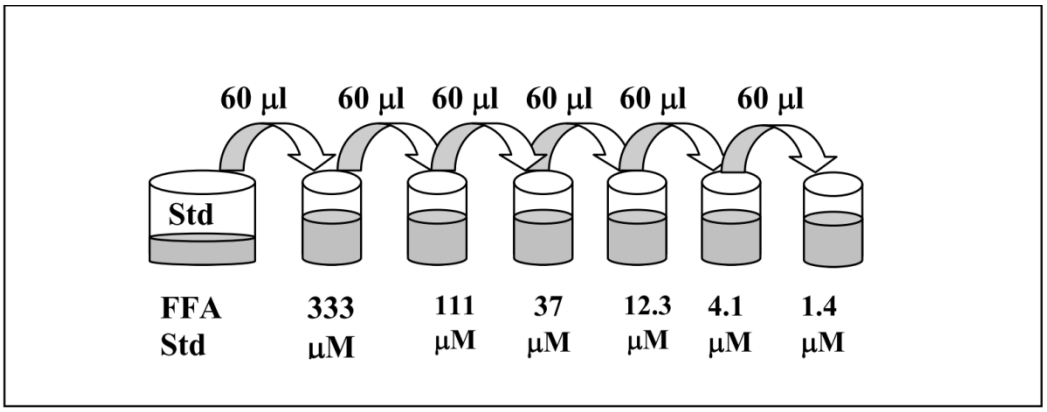
1. Add 5 µl (or 1~10 µl) of serum or plasma sample to a well of Plate A.
2. Add dilution buffer to each well to reach a total sample volume of 50 µl.
3. Addition of 5 µl results in a 10x dilution of sample (5 µl of serum/plasma sample in 50 µl total sample volume).
4. Add 50 µl of each standard to empty wells. Use Plate B if necessary.
5. Add 10.5 ml of the reconstituted FFA Reagent A to one of the disposable trays provided with the kit.
6. Add 100 µl of FFA Reagent A to each well.
7. Gently shake the plate to ensure thorough mixing.
8. Place in a 37$^{o}$C incubator for 10 minutes.
9. Add 5.5 ml of the reconstituted FFA Reagent B to the other disposable tray provided with the kit.
10. Add 50 µl of FFA Reagent B to each well.
11. Gently shake the plate to ensure thorough mixing.
12. Place in a 37$^{o}$C incubator for 10 minutes.
13. Allow the plate to equilibrate to room temperature for 5 minutes. During this time, ensure that there are no bubbles in the solution mixture.
14. Use a large gauge needle or clean pipet tip to pop any bubbles as this will result in inaccurate absorbance readings.
15. Measure the optical density of each well at 540 nm.

# Reagent Preparation: *(This area may have several different preparations with the table of contents below.)*

## Reagent 1:

Preparation of standard curve using the Standard Solution:

1. Briefly spin down the contents of the FFA standard tube before reconstitution.
2. Standard FFA concentrations are 0, 1.4, 4.1, 12.3, 37, 111, and 333 µM.
3. The kit standard solution is the 1.0 mM standard concentration.
4. Pipette 120 µl of Dilution Buffer into 6 tubes.
5. Pipette 60 µl of the FFA Standard Stock solution into a tube labeled 333 µM.
6. Prepare a dilution series as depicted below.
7. Mix each new dilution thoroughly before proceeding to the next solution.
8. The Dilution Buffer alone serves as the zero standard solution.




## Reagent 2:

Preparation of FFA Reagent A:

1. Add 10.5 ml FFA Diluent A per bottle, and gently invert. Do not vortex.
2. Store any remaining solution at 2~8°C. The reagent solution is stable for 10 days after reconstitution when refrigerated at 2~8°C.

## Reagent 3:

Preparation of FFA Reagent A:

1. Add 5.5 ml FFA Diluent B per bottle, and gently invert. Do not vortex.
2. Store any remaining solution at 2~8°C. The reagent solution is stable for 10 days after reconstitution when refrigerated at 2~8°C.