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U Mass - Non-esterified fatty acids

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Protocol status: Working
We use this protocol and it's working

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ABSTRACT

Summary:

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.

MATERIALS

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- ☒ 96-well assay plate blank **Zen-Bio Catalog #SFA-1**
- ☒ Dilution Buffer **Zen-Bio Catalog #SFA-1**
- ☒ FFA Standard **Zen-Bio Catalog #SFA-1**
- ☒ FFA Diluent A **Zen-Bio Catalog #SFA-1**
- ☒ FFA Diluent B **Zen-Bio Catalog #SFA-1**
- ☒ FFA Reagent A **Zen-Bio Catalog #SFA-1**
- ☒ FFA Reagent B **Zen-Bio Catalog #SFA-1**
- ☒ Multichannel Pipette Tray **Zen-Bio Catalog #SFA-1**

Additional Items

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

Reagent Preparation:

Keywords: Non-esterified fatty acids, obesity, insulin resistance, type 2 diabetes

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.

BEFORE START INSTRUCTIONS

Notes:

✓ Freshly prepared blood or plasma samples are recommended. If storing samples, keep blood and plasma samples at -20°C or at -70°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°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°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.