Product Manual

Free Fatty Acid Assay Kit (Colorimetric)

Catalog Number

STA-618 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

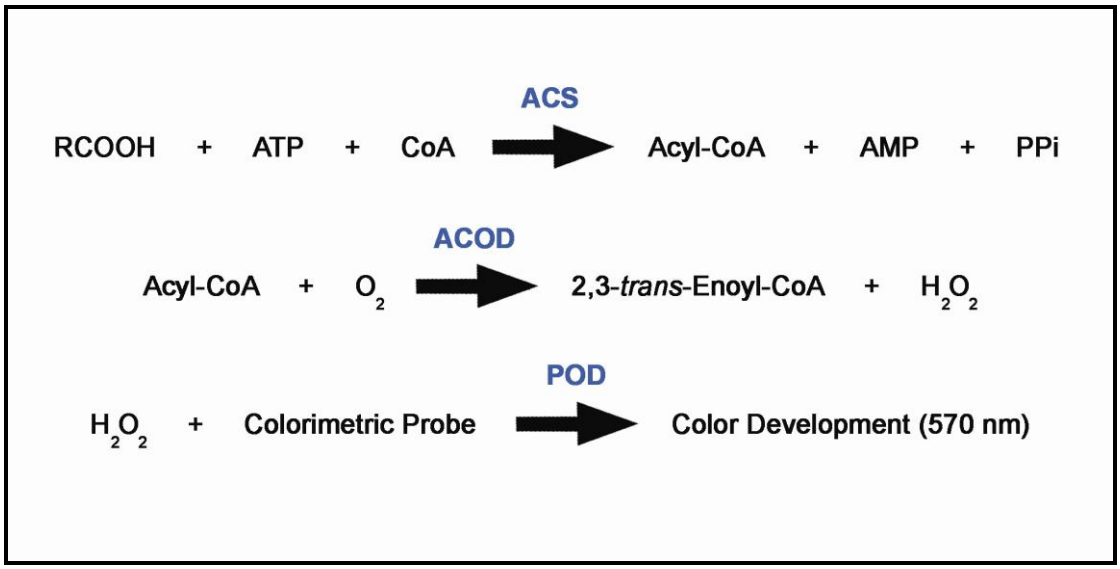
Triglycerides (TAG) are a type of lipid in the blood, serving as an energy source and playing a key role in metabolism. Triglycerides are the digestive end product of breaking down dietary fats. Any extra carbohydrates and fats that are not immediately used are chemically converted into triglycerides. In the intestines, secreted enzyme lipases hydrolyze the triglyceride ester bond, yielding glycerol and free fatty acids (FFA) in a process called lipolysis. Additionally, hormones induce and regulate lipase activity in adipose tissue, resulting in changes to blood FFA levels. Free fatty acids then bind plasma albumin for circulation in the body, serving as a readily absorbed energy source for muscle, brain and other organ tissues. Measurement of free fatty acids has become useful in monitoring and diagnosis of several diseases and metabolic disorders (e.g. obesity, insulin resistance, diabetes, cancer).

Cell Biolabs' Free Fatty Acid Assay Kit measures non-esterified fatty acids (NEFA) in serum and plasma by a coupled enzymatic reaction system (ACS-ACOD Method). First, Acyl CoA Synthetase (ACS) catalyzes fatty acid acylation of coenzyme A. Next, the acyl-CoA product is oxidized by Acyl CoA Oxidase (ACOD), producing hydrogen peroxide which reacts with the kit's Colorimetric Probe (absorbance maxima of 570 nm).

The Free Fatty Acid Assay Kit is a simple, colorimetric assay that quantitatively measures the free fatty acid concentration (non-esterified) in various samples using a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, standards and unknown samples. The kit contains a palmitic acid standard and has a detection sensitivity limit of ~15 µM.

Note: This kit is not suitable for urine or heparin-containing samples. Fatty acids (C8 and longer) can be quantified with this kit.

Assay Principle



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5. STA-375: Uric Acid/Uricase Assay Kit
6. STA-378: Creatinine Assay Kit
7. STA-390: Total Cholesterol Assay Kit
8. STA-391: HDL and LDL/VLDL Cholesterol Assay Kit
9. STA-394: HDL Cholesterol Assay Kit
10. STA-396: Serum Triglyceride Quantification Kit (Colorimetric)
11. STA-397: Serum Triglyceride Quantification Kit (Fluorometric)
12. STA-398: Free Glycerol Assay Kit (Colorimetric)
13. STA-399: Free Glycerol Assay Kit (Fluorometric)

Kit Components

1. FFA Standard (Part No. 261801): One 100 µL vial of 50 mM palmitic acid in ethanol.
2. 20X Assay Buffer (Part No. 261802): One 1.5 mL vial.
3. 5X Enzyme Mixture A (Part No. 261803): Four 1 mL vials (containing ACS, Ascorbate Oxidase, and necessary cofactors).
4. 5X Enzyme Mixture B (Part No. 261804): Four 0.5 mL vials (containing ACOD).
5. NEM Reagent (Part No. 261805): One 150 µL amber vial.
6. Colorimetric Probe (Part No. 261806): One 110 µL amber vial.

Materials Not Supplied

1. 96-well microtiter plate
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate reader capable of reading at 570 nm

Storage

Store the entire kit at -80ºC. Avoid multiple freeze/thaws by aliquoting. The NEM Reagent and Colorimetric Probe are light sensitive and should be maintained in amber tubes.

Preparation of Reagents

- FFA Standard: Thaw at 37ºC for 10 minutes. Once homogeneous and mixed well, maintain the standard at room temperature during assay preparation. The solution is stable for 1 week at room temperature. For longer term storage, the solution should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.
- 1X Assay Buffer: 20X Assay Buffer should be thawed/maintained at 4ºC during assay preparation. Dilute the 20X Assay Buffer with deionized water. Stir to homogeneity. The 1X solution is stable for 1 week at 4ºC. For longer term storage, any unused 20X stock material should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.
- 1X Enzyme Mixture (A or B): Each 5X Enzyme Mixture should be thawed/maintained at 4ºC during assay preparation. Dilute the 5X Enzyme Mixture with cold, deionized water. Stir to homogeneity. The 1X solution is stable for 1 week at 4ºC. For longer term storage, any unused 5X stock material should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.

  Note: These components are provided in multiple tubes to minimize multiple freeze/thaws.

- NEM Reagent: Thaw and maintain at 4ºC during assay preparation. The solution is stable for 1 week at 4ºC. For longer term storage, the solution should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.
- Colorimetric Probe: Thaw and maintain at room temperature during assay preparation. Any unused material should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.

Preparation of FFA Standard

Heat the FFA Standard at 37ºC for 10 minutes. Mix well by vortexing to ensure the solution is homogeneous and free of palmitic acid crystals. Freshly prepare a dilution series of standard in the concentration range of 0 µM – 500 µM by diluting the standard stock solution (provided at 50 mM) in 1X Assay Buffer (see Table 1). FFA diluted solutions and standards should be prepared fresh, vortexed well and used immediately.

Standard Tubes	50 mM FFA Standard (µL)	1X Assay Buffer (µL)	Final FFA Standard (µM)
1	10	990	500
2	500 of Tube #1	500	250
3	500 of Tube #2	500	125
4	500 of Tube #3	500	62.5
5	500 of Tube #4	500	31.25
6	500 of Tube #5	500	15.63
7	500 of Tube #6	500	7.81
8	0	500	0

Table 1. Preparation of Free Fatty Acid Standards

Preparation of Samples

- Urine: This kit is not recommended for urine samples**.**
- Plasma: Collect blood with an anticoagulant such as citrate, EDTA or oxalate and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer. Sample should be tested immediately or frozen at -80°C for storage. Plasma must be diluted before assaying (1:2 to 1:20 in 1X Assay Buffer). Normal FFA levels in human plasma are typically 150-450 µM.

  Note: Heparin is known to interfere with the assay. Heparin-containing samples including heparinized plasma should be avoided.
- Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C for storage. Serum must be diluted before assaying (1:2 to 1:20 in 1X Assay Buffer). Normal FFA levels in human serum are typically 100-700 µM.

Assay Protocol

Each FFA standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add 10 µL of each FFA standard, sample and blank to the 96-well microtiter plate.
2. Add 200 µL of 1X Enzyme Mixture A (see Preparation of Reagents) to each well.
3. Cover the plate wells to protect the reaction from light.
4. Incubate at 37ºC for 30 minutes.
5. During the step 4 incubation, separately prepare the desired volume of Detection Enzyme Mixture according to Table 2 below, based on the number of tests to be performed. Maintaining all components and mixtures at 4ºC throughout this step, add components in the following sequence:
   a. In a tube, add the appropriate volume of 1X Enzyme Mixture B (see Preparation of Reagents).
   b. To the 1X Enzyme Mixture B add the corresponding volume of NEM Reagent. Mix well.
   c. Finally, add the corresponding volume of Colorimetric Probe. Mix well and use immediately.

   Note: Detection Enzyme Mixture will appear slightly pink in color. This is normal background and should be subtracted from all absorbance values.

1X Enzyme Mixture B (mL)	NEM Reagent (µL)	Colorimetric Probe (µL)	Total Volume of Detection Enzyme Mixture (mL)	**# of Tests in 96-well Plate (100 µL/test)**
10	100	100	10.2	**100**
5	50	50	5.1	**50**
2.5	25	25	2.55	**25**

Table 2. Preparation of Detection Enzyme Mixture

6. Transfer 100 µL of the above Detection Enzyme Mixture to each well (from step 4).
7. Cover the plate wells to protect the reaction from light.
8. Incubate at 37ºC for 10 minutes.
9. Read absorbance at 570 nm on a microplate reader.
10. Calculate the concentration of free fatty acid within samples by comparing the sample absorbance to the standard curve. Negative controls (without FFA) should be subtracted.

Example of Results

The following figures demonstrate typical Free Fatty Acid Assay Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

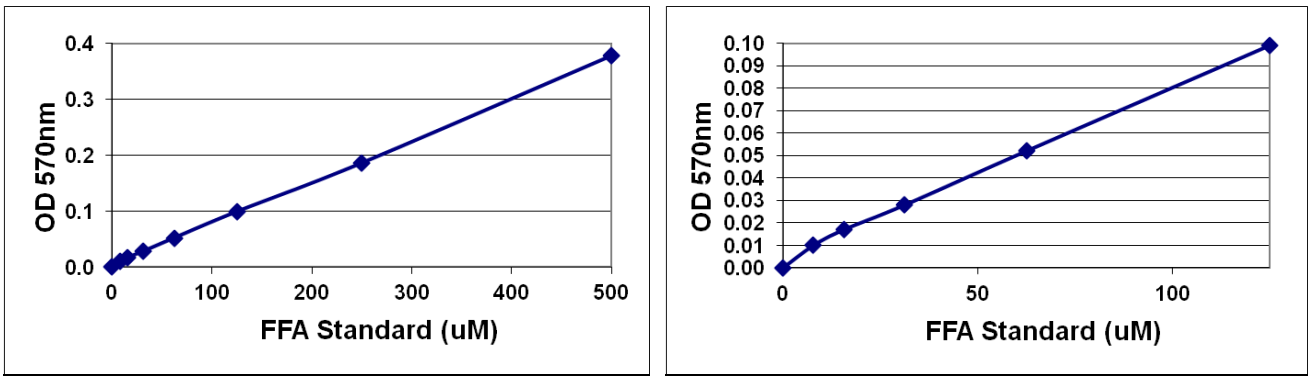

Figure 1: Free Fatty Acid Assay Standard Curve. FFA standard curve was performed according to the Assay Protocol. Background has been subtracted.

References

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Recent Product Citation

Diané, A. et al. (2014). PACAP is essential for the adaptive thermogenic response of brown adipose tissue to cold exposure. *J Endocrinol*. **222**:327-339.

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