Free Fatty Acid Assay Kit

Free Fatty Acid Assay Kit is a detection kit for the quantification of Free Fatty Acid in serum, plasma, urine, saliva, milk, cell cultures and food.

Catalog number: ARG82005

Package: 100 tests

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INTRODUCTION

In chemistry, particularly in biochemistry, a fatty acid is a carboxylic acid with a long aliphatic chain, which is either saturated or unsaturated. Most naturally occurring fatty acids have an unbranched chain of an even number of carbon atoms, from 4 to 28. Fatty acids are a major component of the lipids (up to 70 wt%) in some species such as microalgae but in some other organisms are not found in their standalone form, but instead exist as three main classes of esters: triglycerides, phospholipids, and cholesteryl esters. In any of these forms, fatty acids are both important dietary sources of fuel for animals and important structural components for cells. [Provide by Wikipedia: Fatty Acid]

PRINCIPLE OF THE ASSAY

This Free Fatty Acid Assay Kit is a simple assay that measures the amount of Free Fatty Acid in in biological samples such as serum, plasma, urine, saliva, milk, cell cultures and food. In this assay, free fatty acids are enzymatically converted to acyl-CoA and subsequently to H_2O_2. The resulting H_2O_2 reacts with a specific dye to form a pink colored product. The optical density at O.D. 570 nm or fluorescence intensity (λex/em = 530/585 nm) is directly proportional to the free fatty acid concentration in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped on ice. Store all reagent at-20°C upon receiving. Shelf life: 6 months after receipt.

Component	Quantity	Storage information
Assay Buffer	20 mL	-20°C
Enzyme A, Lyophilized	1 vial	-20°C
Enzyme B	120 µL	-20°C
CoSubstrate	120 µL	-20°C
Dye Reagent	120 µL	-20°C
Standard (1 mM palmitic acid)	1 mL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 570 nm
- Fluorescence microplate reader capable of reading excitation at 530 nm and emission at 585 nm
- Centrifuge and centrifuge tube
- Clear or black flat-bottom 96 well microplate
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- SH-containing reagents (E.g., β–mercaptoethanol, dithiothreitol > 5 µM), sodium azide, EDTA, and sodium dodecyl sulfate are known to interfere in this assay and should be avoided in sample preparation.
- The thawed Standard solution should be clear and colorless. If the CoSubstrate is turbid, bring it to 37°C and gently swirl the tube (do not vortex) until the solution is clear.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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 at 4°C. Collection the supernatant for assay.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Collection the supernatant for assay.

Urine: assayed directly. If particulates are present, centrifuge sample (5 minutes at 2,000 x g) and use the clear supernatant for the assay.

Milk and solid sample: sample can be homogenized in 5% isopropanol and 5% Triton X-100 in distilled water, followed by filtration through a 0.45 µm PTFE syringe filter.

Note:

- SH-containing reagents (E.g., β–mercaptoethanol, dithiothreitol > 5 µM), sodium azide, EDTA, and sodium dodecyl sulfate are known to interfere in this assay and should be avoided in sample preparation.

REAGENT PREPARATION

- **Reconstitute Enzyme A:** adding 120 µL of distilled water to the Enzyme A tube. Make sure Enzyme A is fully dissolved by pipetting up and down and incubate at RT for 15 minutes. Store reconstituted Enzyme A at -20°C and use within 2 months.
- **Working Reagent:** for each reaction, mix 90 µL of Assay Buffer, 1 µL of Reconstitute Enzyme A, 1 µL of Enzyme B, 1 µL of CoSubstrate and 1 µL of Dye Reagent.
- **Standard:** Dilute Standard in Assay Buffer as follows:

Standard tube	Palmitic Acid (µM)	Assay Buffer (µL)	Standard, 1000 µM (µL)
S1	1000	0	100
S2	600	40	60
S3	300	70	30
S4	0	1000	0

Note: the thawed Standard solution should be clear and colorless.

ASSAY PROCEDURE

Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening. During experiment, keep thawed tubes in a refrigerator or on ice.

COLORIMETRIC PROCEDURE (clear flat-bottom 96 well plate)

	Standard well	Sample well
Each diluted Standard	10 µL	
Each Sample		10 µL
Working Reagent	90 µL	90 µL
Tap plate to mix briefly and thoroughly. Incubate for **30 minutes** at **room temperature**.		
Read the absorbance at **O.D. 570 nm** (550-585 nm).		

FLUORIMETRIC PROCEDURE (black flat-bottom 96 well plate)

For fluorimetric assays, the linear detection range is 0, 30, 60 and 100 µM Standard. Dilute the standards from COLORIMETRIC PROCEDURE 10X with Assay Buffer. Final read fluorescence intensity at **λex/em** = **530/585 nm**.

CALCULATION OF RESULTS

1. Subtract blank value (Standard tube, S4) from the standard values and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the fatty acid concentration of Sample,

   Free Fatty Acid (µM) = [(R_{Sample}-R_{Blank}) / Slope (μM^{-1})] x n

   Note:

   - R_{Sample} and R_{Blank}: the optical density or fluorescent values of the sample and blank, respectively.
   - n: the sample dilution factor.

2. If the calculated free fatty acid concentration of a sample is higher than 1000 µM in the Colorimetric Assay or 100 µM in the Fluorimetric Assay, dilute sample in Assay Buffer and repeat the assay. Multiply result by the dilution factor n.

EXAMPLE OF TYPICAL STANDARD CURVE

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