EnzyChrom™ Free Fatty Acid Assay Kit (EFFA-100)

Quantitative Colorimetric/Fluorimetric Fatty Acid Determination

DESCRIPTION

Fatty acids are aliphatic monocarboxylic acids that are ubiquitously found in animal or vegetable fat, oil and wax. Fatty Acids play important roles in cellular synthesis, energy metabolism and are implicated in diverse disorders such as diabetes mellitus, sudden infant death syndrome and Reye Syndrome. BioAssay Systems' method provides a simple, one-step and high-throughput assay for measuring free fatty acids. 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 570nm or fluorescence intensity (530/585 nm) is directly proportional to the free fatty acid concentration in the sample.

KEY FEATURES

Sensitive. Use 10 μL samples. Linear detection range: colorimetric assay 7 - 1000 μM, fluorimetric assay 7 - 100 μM fatty acid.

Convenient. Room temperature "mix-and-read" procedure can be readily automated for high-throughput assay of thousands of samples per day.

APPLICATIONS

Assays: free fatty acids in biological samples such as serum, plasma, urine, saliva, milk, cell cultures and in food, agriculture products.
Drug Discovery/Pharmacology: effects of drugs on free fatty acid metabolism.

KIT CONTENTS

Assay Buffer:	20 mL	**Dye Reagent:**	120 μL
Enzyme A:	Dried	**Enzyme B:**	120 μL
CoSubstrate:	120 μL	**Standard**:	1 mL 1 mM palmitic acid

Storage conditions. The kit is shipped on ice. Store all components at -20°C. Shelf life of six months after receipt.

Precautions: 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.

PROCEDURES

Reagent Preparation:

Reconstitute Enzyme A by adding 120 μL dH_2O to the Enzyme A tube. Make sure Enzyme A is fully dissolved by pipetting up and down and incubate at RT for 15 min. Store reconstituted Enzyme A at -20°C and use within 2 months.

Colorimetric Assay:

Liquid samples such as serum and plasma can be assayed directly. Milk and solid samples can be homogenized in 5% isopropanol and 5% Triton X-100 in water, followed by filtration through a 0.45μm PTFE syringe filter (e.g. VWR Cat# 28145-493).

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.

1. Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening. Keep thawed tubes on ice during assay.
   Important: the thawed Standard solution should be clear and colorless. If the Substrate is turbid, bring it to 37°C and gently swirl the tube (do not vortex) until the solution is clear.
2. *Standards*: Dilute standard in Assay Buffer as follows.

No	1000 μM STD + Buffer	Vol (μL)	Palmitic Acid (μM)
1	100 μL + 0 μL	100	1000
2	60 μL + 40 μL	100	600
3	30 μL + 70 μL	100	300
4	0 μL +100 μL	100	0

   Transfer 10 μL diluted standards into separate wells of a clear flat-bottom 96-well plate.

   Samples: transfer 10 μL of each sample into separate wells of the plate.
3. *Color reaction*. Prepare enough Working Reagent by mixing, for each well, 90 μL Assay Buffer, 1 μL Enzyme A, 1 μL Enzyme B, 1 μL CoSubstrate and 1 μL Dye Reagent. Add 90 μL Working Reagent to each well. Tap plate to mix. Incubate 30 min at room temperature.
4. Read optical density at 570nm (550-585nm).

Fluorimetric Assay:

The fluorimetric assay procedure is similar to the colorimetric procedure except that (1) 0, 30, 60 and 100 μM Standards and (2) a black 96-well plate are used. Read fluorescence intensity at λ_{ex} = 530 nm and λ_{em} = 585 nm.

Note: 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.

CALCULATION

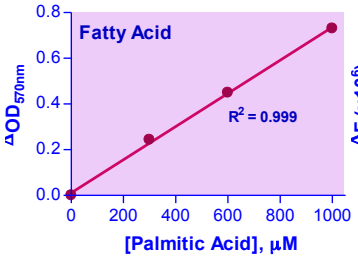
Subtract blank value (#4) from the standard values and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the fatty acid concentration of Sample,

$$[\text{Free Fatty Acid}] = \frac{R_{SAMPLE} - R_{BLANK}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M)$$

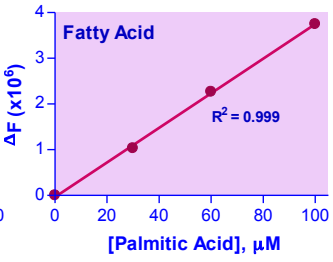
R_{SAMPLE} and R_{BLANK} are optical density or fluorescence intensity readings of the Sample and Buffer Blank, respectively. n is the sample dilution factor.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom uncoated 96-well plates (e.g. VWR cat# 82050-760), optical density plate reader; black flat-bottom uncoated 96-well plates (e.g. VWR cat# 82050-676), fluorescence plate reader. For milk and solid samples, 0.45μm PTFE syringe filter and 5% isopropanol, 5% Triton X-100 solution.



96-well colorimetric assay



96-well fluorimetric assay

PUBLICATIONS

1. Hwang I et al (2012) Catalase deficiency accelerates diabetic renal injury through peroxisomal dysfunction. Diabetes 61(3):728-738.
2. Jelinek D et al (2012) The Niemann-Pick C1 gene is downregulated in livers of C57BL/6J mice by dietary fatty acids, but not dietary cholesterol, through feedback inhibition of the SREBP pathway. J Nutr 142(11):1935-1942.
3. Seo, CW et al (2011). Antihyperlipidemic and body fat-lowering effects of silk proteins with different fibroin/sericin compositions in mice fed with high fat diet. J Agric Food Chem 59(8):4192-4197.