Free Fatty Acid Assay Kit (Colorimetric/Fluorometric)

LS-K170-100 (100 Tests) • **Store at -20°C**

Introduction

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. LSBio's 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, fluorometric 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.

Components

Component	K170-100
	100 Tests
Assay Buffer	20 mL
Enzyme A	Dried
Enzyme B	120 µL
Dye Reagent	120 µL
CoSubstrate	120 µL
Standard (1 mM palmitic acid)	1 mL

Materials Not Supplied

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.

Storage

The kit is shipped on ice. Store all kit components at -20 °C.

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Assay Procedure

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).

Fluorometric Assay

The fluorometric 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 λex = 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 Fluorometric Assay, dilute sample in Assay Buffer and repeat the assay. Multiply result by the dilution factor *n*.

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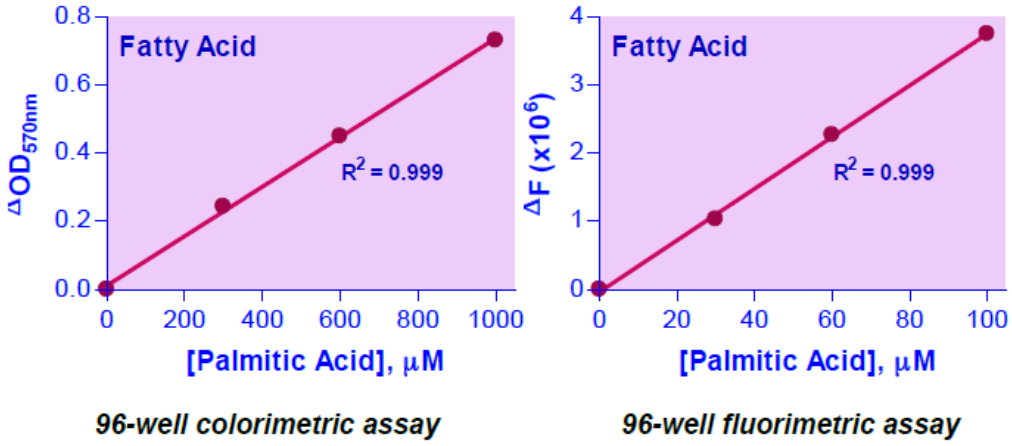
Calculation

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,

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

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.

Sample Data



96-well colorimetric assay *96-well fluorimetric assay*

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