Technical Bulletin

Free Fatty Acid Assay Kit

Catalogue Number MAK466

Product Description

Fatty acids are aliphatic monocarboxylic acids 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.

The Free Fatty Acid Assay Kit 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 570 nm or fluorescence intensity (λ_{Ex} = 530 nm/λ_{Em} = 585 nm) is directly proportional to the free fatty acid concentration in the sample.

The linear detection range for the kit is 7-1000 µM for the colorimetric assay and 7-100 µM for the fluorometric assay. The kit is suitable for detecting free fatty acids in biological samples such as serum, plasma, urine, saliva, milk, and cell cultures, and in food and agriculture products, as well as studying the effects of drugs on free fatty acid metabolism.

Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- Assay Buffer — 20 mL
  Catalogue Number MAK466A
- Dye Reagent — 120 µL
  Catalogue Number MAK466B
- Enzyme A — 1 vial
  Catalogue Number MAK466C
- Enzyme B — 120 µL
  Catalogue Number MAK466D
- CoSubstrate — 120 µL
  Catalogue Number MAK466E
- Standard (1 mM palmitic acid) — 1 mL
  Catalogue Number MAK466F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (such as, multichannel pipettor)
- 1.5 mL microcentrifuge tubes
- Multiwell plate reader
- Clear flat-bottom 96-well plates for colorimetric assay or black flat-bottom 96-well plates for fluorometric assay. Cell culture or tissue culture treated plates are **not** recommended.
- Dounce tissue grinder set (for milk and solid samples only)
  (Catalogue Number D9063 or equivalent)
- 0.45 µm PTFE syringe filter (for milk and solid samples only) (Catalogue Number SLCRX13 or equivalent)
- 5% isopropanol (for milk and solid samples only) (Catalogue Number 190764 or equivalent)
- Triton™ X-100 (for milk and solid samples only) (Catalogue Number X100 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

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

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use. Keep thawed tubes on ice during assay.

Important: The thawed Standard solution should be clear and colorless. If the Standard is turbid, bring it to 37 °C and gently swirl the tube (do not vortex) until the solution is clear.

Enzyme A: Reconstitute the vial with 120 µL of purified water. Make sure Enzyme A is fully dissolved by pipetting up and down and incubate at room temperature for 15 minutes. Store reconstituted Enzyme A solution at -20 °C and use within 2 months.

Procedure

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

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 purified water, followed by filtration through a 0.45 µm PTFE syringe filter.

Transfer 10 µL of each sample into separate wells of the plate.

Colorimetric Standard Curve Preparation

1. Using the 1 mM (1000 µM) Palmitic Acid Standard, prepare standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.
Preparation of Colorimetric Standards

Well	1000 µM Standard	Assay Buffer	Palmitic Acid (µM)
1	100 µL	-	1000
2	60 µL	40 µL	600
3	30 µL	70 µL	300
4	-	100 µL	0

2. Mix well and transfer 10 µL of each Standard into separate wells of a clear 96-well plate.

Fluorometric Standard Curve Preparation

1. Using the 1 mM (1000 µM) Palmitic Acid Standard, prepare standards in 1.5 mL microcentrifuge tubes according to Table 2.

Table 2.
Preparation of Fluorometric Standards

Well	1000 µM Standard	Assay Buffer	Palmitic Acid (µM)
1	10 µL	90 µL	100
2	6 µL	94 µL	60
3	3 µL	97 µL	30
4	-	100 µL	0

2. Mix well and transfer 10 µL of each Standard into separate wells of a black 96-well plate.

Working Reagent

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 94 µL of Working Reagent according to Table 3.

Table 3.
Preparation of Working Reagents

Reagent	Volume
Assay Buffer	90 µL
Enzyme A	1 µL
Enzyme B	1 µL
CoSubstrate	1 µL
Dye Reagent	1 µL

2. Transfer 90 µL of Working Reagent into each standard and sample well. Tap plate to mix.

Measurement

Incubate at room temperature for 30 minutes. Measure the absorbance (OD) at 570 nm or fluorescence (F) at λ_{Ex} = 530 nm/ λ_{Em} = 585 nm.

Results

1. Subtract the blank (Standard #4) absorbance (OD) or fluorescence (F) value from the remaining standard values.
2. Plot the ΔOD or ΔF against the Standard concentrations.
3. Determine the slope of the standard curve and calculate the fatty acid concentration of Sample.

   Free Fatty Acid (µM) =

$$\frac{R_{SAMPLE} - R_{BLANK}}{Slope\ (\mu M^{-1})}\ x\ DF$$

where:

R_{Sample} = Absorbance (OD) or fluorescence (F) values of Sample

R_{Blank} = Absorbance (OD) or fluorescence (F) values of Blank

DF = Sample dilution factor (DF = 1 for undiluted Samples)

If the calculated free fatty acid concentration of a Sample is higher than the 1000 µM Standard in the colorimetric assay or the 100 µM Standard in the fluorometric assay, dilute the Sample in Assay Buffer and repeat the assay. Multiply the result by the dilution factor (DF).

Figure 1.
Typical Standard Curve (Colorimetric Assay)

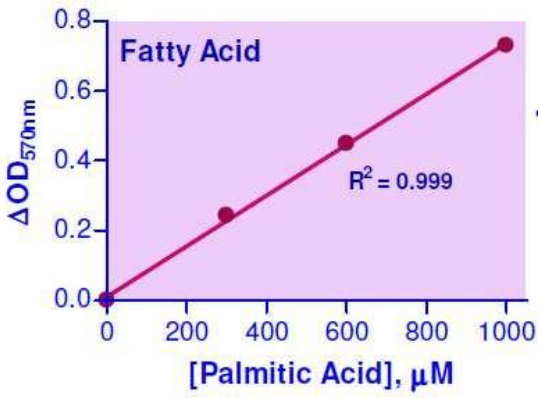
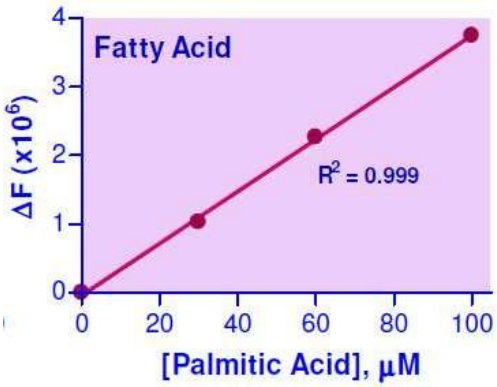


Figure 2.
Typical Standard Curve (Fluorometric Assay)



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