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Product Information

Free Fatty Acid Quantitation Kit

Catalog Number **MAK044**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Free fatty acids, also know as nonesterified fatty acids, circulate in the plasma bound to albumin. The majority of free fatty acids are derived from either dietary sources or are mobilized from adipose tissue. Plasma free fatty acids are elevated in many obesity-related disorders and may contribute to insulin resistance in peripheral tissues. Conditions such as sepsis and tumors producing lipoactive homones may also be associated with elevated free fatty acid levels.

In this kit, the concentration of fatty acids (C8 and longer) is determined by a coupled enzyme assay, which results in a colorimetric (570 nm)/ fluorometric (λ_{ex} = 535/λ_{em} = 590 nm) product, proportional to the fatty acids present.

Components

The kit is sufficient for 100 assays in 96 well plates.

Fatty Acid Assay Buffer Catalog Number MAK044A	25 mL
Fatty Acid Probe in DMSO Catalog Number MAK044B	0.2 mL
ACS Reagent Catalog Number MAK044C	1 vl
Enzyme Mix Catalog Number MAK044D	1 vl
Enhancer Catalog Number MAK044E	0.2 mL
Palmitic Acid Standard, 1 nmole/µL Catalog Number MAK044F	0.3 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader
- Chloroform (Catalog Number C2432 or equivalent) and Triton™ X-100 (Catalog Number T9284 or equivalent)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Fatty Acid Assay Buffer – Allow buffer to come to room temperature before use.

Fatty Acid Probe – Warm to room temperature to thaw the solution prior to use. Store protected from light and moisture at –20 °C. Upon thawing, the Fatty Acid Probe is ready-to-use in the colorimetric assay. Use within 2 months.

For the fluorescence assay, dilute an aliquot of the Fatty Acid Probe Solution 5 to 10-fold with Fatty Acid Assay Buffer, just prior to use. This will reduce the background of the fluorescence assay.

ACS (Acyl-CoA Synthetase) Reagent and Enzyme Mix – Reconstitute each with 220 µL of Fatty Acid Assay Buffer. Mix well by pipetting (don't vortex), then aliquot and store, protected from light, at –20 °C. Use within 2 months of reconstitution.

Palmitic Acid Standard – If separation from the aqueous phase occurs after being frozen, the Palmitic Acid Standard will need to be re-dissolved. Place in hot water bath (80-100 °C) for 1 minute, then vortex for 30 seconds. Once the standard is clear in color, repeat water bath and vortex again.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure

All samples and standards should be run in duplicate.

Palmitic Acid Standards for Colorimetric Detection

Add 0, 2, 4, 6, 8, and 10 µL of the standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Fatty Acid Assay Buffer to each well to bring the volume to 50 µL.

Palmitic Acid Standards for Fluorometric Detection

Dilute 10 µL of the 1 nmole/µL standard solution with 90 µL of the Fatty Acid Assay Buffer to prepare a 0.1 nmole/µL standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 0.1 nmole/µL standard solution into a 96 well plate, generating 0 (blank), 0.2, 0.4, 0.6, 0.8, and 1 nmole/well standards. Add Fatty Acid Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation

Tissue (10 mg) or cells (1×10^6) can be homogenized in 200 µL of a 1% (w/v) Triton X-100 in chloroform solution. Centrifuge the samples at 13,000 $\times$ *g* for 10 minutes to remove insoluble material. Collect the organic phases (lower phase) and air dry at 50 °C to remove chloroform. Vacuum dry for 30 minutes to remove trace chloroform. Dissolve the dried lipids in 200 µL of Fatty Acid Assay Buffer by vortexing extensively for 5 minutes. The solution may be turbid or cloudy.

Serum and other liquid samples can be directly added to wells.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Bring samples to a final volume of 50 µL with Fatty Acid Assay Buffer.

Assay Reaction

1. Add 2 µL of ACS Reagent to each sample and standard well, and incubate for 30 minutes at 37 °C.

2. Set up the Master Reaction Mix according to the scheme in Table 1. 50 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Volume
Fatty Acid Assay Buffer	44 µL
Fatty Acid Probe	2 µL
Enzyme Mix	2 µL
Enhancer	2 µL

3. Add 50 µL of the Master Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at 37 °C. Protect the plate from light during the incubation.

4. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (λ_{ex} = 535/λ_{em} = 590 nm).

Results

Calculations

The background for either assay is the value obtained for the 0 (blank) palmitic acid standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings.

Use the values obtained from the appropriate palmitic acid standards to plot a standard curve. The amount of fatty acids present in the samples may be determined from the standard curve.

Note: A new standard curve must be set up each time the assay is run.

Concentration of Fatty Acids

$$F_a/S_v = C$$

F_a = Amount of Fatty Acids in unknown sample (nmole) from standard curve

S_v = Sample volume (µL) added to reaction well

C = Concentration of Fatty Acids in sample

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer to and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the Reaction Mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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