**Product Information**

**Free Fatty Acid Quantitation Kit**

Catalog Number MAK044  
Storage Temperature −20 °C

**TECHNICAL BULLETIN**

**Product Description**

Free fatty acids, also known 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 hormones 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 (λex = 535/λem = 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 ml

- Enzyme Mix  
  Catalog Number MAK044D  
  - 1 ml

- 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 × 10⁶) can be homogenized in 200 μL of a 1% (w/v) Triton X-100 in chloroform solution. Centrifuge the samples at 13,000 × 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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty Acid Assay Buffer</td>
<td>44 μL</td>
</tr>
<tr>
<td>Fatty Acid Probe</td>
<td>2 μL</td>
</tr>
<tr>
<td>Enzyme Mix</td>
<td>2 μL</td>
</tr>
<tr>
<td>Enhancer</td>
<td>2 μL</td>
</tr>
</tbody>
</table>

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 (A₅₇₀). For fluorometric assays, measure fluorescence intensity (λₑₓ = 535/λₑₘ = 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

\[ \frac{F_a}{S_v} = C \]

\[ F_a = \text{Amount of Fatty Acids in unknown sample (nmole)} \]

\[ S_v = \text{Sample volume (μL) added to reaction well} \]

\[ C = \text{Concentration of Fatty Acids in sample} \]
# Troubleshooting Guide

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

Triton is a trademark of The Dow Chemical Company or an affiliated company of Dow.