

# Carboxylic Acid MS Labeling Kit (CM63401) User Reference Guide

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## Important Notes & Contact Information

### READ BEFORE USING ANY RESOURCES PROVIDED HEREIN

The information provided in this document and the methods included in this package are for information purposes only. CellMosaic provides no warranty of performance or suitability for the purpose described herein. The performance of labeling using this kit may be affected by many different variables, including but not limited to: purity and complexity of the molecules, differences in preparation techniques, operator abilities, and environmental conditions.

Sample data are provided for illustration and example purposes only and represent a small dataset used to verify kit performance in the CellMosaic laboratory. Information about the chemicals and reagents used in the kit are provided as necessary.

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## Kit Components

This kit provides materials to perform quaternary amine (MS-Tags) labeling of carboxylic acids, including fatty acids and prostaglandins, for enhanced detection by positive ion mode mass spectrometric analysis. The kit contains reagents for a total of 50 labeling reactions for light quaternary amine (LQA) and heavy quaternary amine (HQA).

Name	Part #	Quantity	Storage condition	Assay
Light Quaternary Amine (LQA)	CM63001	2 X 1 mL (6 mM)	-20°C/dry	25 labeling reactions per tube, total 50
Heavy Quaternary Amine (HQA)	CM63002	2 X 1 mL (6 mM)	-20°C/dry	25 labeling reactions per tube, total 50
Reagent A	CM10006	2 x 0.5 mL	-20°C/dry	50 labeling reactions per tube, total 100

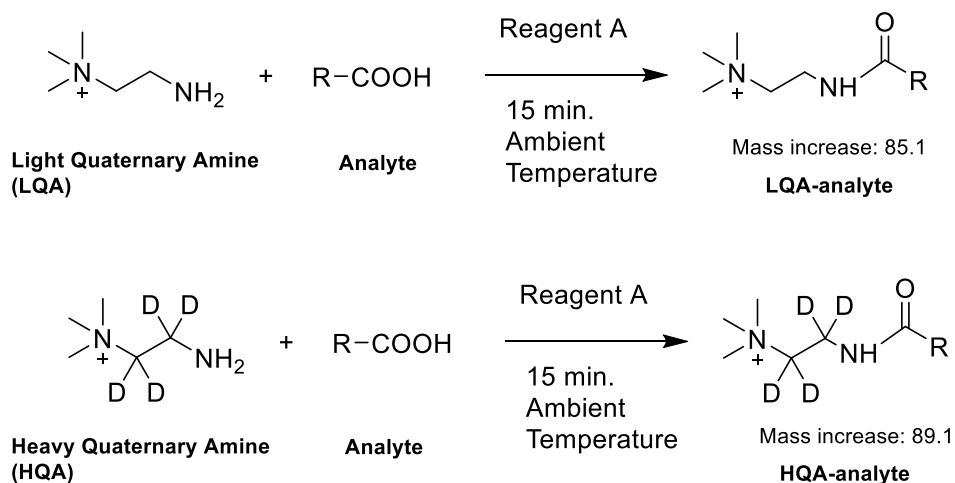
## Safety Information

Warning: some of the chemicals used can be potentially hazardous and cause injury or illness. Please read and understand the Material Safety Data Sheets (MSDS) available at CellMosaic.com before you store, handle, or use any of the materials.

## Labeling Chemistry

Carboxylic acid-containing molecules, including fatty acids and prostaglandins, play key roles in metabolic pathways. Detection and quantitation of such molecules by mass spectrometry after derivatization with quaternary amine containing signal-enhancing molecules is well documented in the literature (Torde *et al.* **2013** Multiplexed Analysis of Cage and Cage Free Chicken Egg Fatty Acids Using Stable Isotope Labeling and Mass Spectrometry. *Molecules*, 18(12), 14977–14988).

CellMosaic designed this kit for simple one step derivatization of carboxylic acid. After 10-minute ambient temperature derivatization, the reaction can be analyzed directly by LC-MS techniques. The reaction scheme is shown below (Scheme 1).



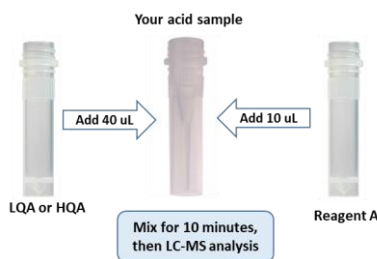
(Nominal mass increase will be slightly different depending on the analyte)

**Scheme 1.** Derivatization Reaction of Carboxylic Acid

## Key Features

1. Simple protocol with less than 15 min total hands-on and reaction time.
2. Quantitative labeling of the acid.
3. 'HQA-labeled analyte' can be used as an internal standard when heavy atom-labeled analyte is not commercially available.
4. Separately, LQA and HQA can be used to label different samples. After quenching the reaction with water, the reactions can be mixed and analyzed in a single LC-MS run to increase the throughput.
5. In MS/MS mode, after neutral loss of trimethyl amine (Me<sub>3</sub>N), the product ion will retain the heavy atoms, enabling different non-interfering parent and product ion monitoring for LQA-Analyte and HQA-Analyte in LC-MS analysis.

## Protocol



### 1. Lab Instrumentation Needed for Derivatization

- Vortex mixer, mini centrifuge
- Pipettes and tips
- Eppendorf tube

## 2. Derivatization Reaction

- a. After sample preparation, dry down the analyte-containing fraction in an Eppendorf tube using speed vacuum concentration [preferable] or nitrogen gas purging.

**Tip for drying:** Ensure your analyte is stable under drying conditions.

- b. Add 40  $\mu$ L of LQA or HQA reagent and 10  $\mu$ L of **Reagent A** into the Eppendorf tube containing the analyte.

**Scalability:** Preferable range for the total amount of carboxylic acid(s) to be labeled is < 150 nmol for quantitative reactions. Please scale up reagent volumes if > 150 nmol of acid is to be labeled.

- c. Mix the tube contents using a vortex mixer for 15-30 seconds and wait for 10 minutes at ambient temperature.
- d. Analyze the reaction mixture using your optimized LC-MS method.

**Tip for analysis:** The reaction mixture contains 100% organic solvent. Dilution with water may be necessary before LC-MS analysis, especially for low retaining compounds.

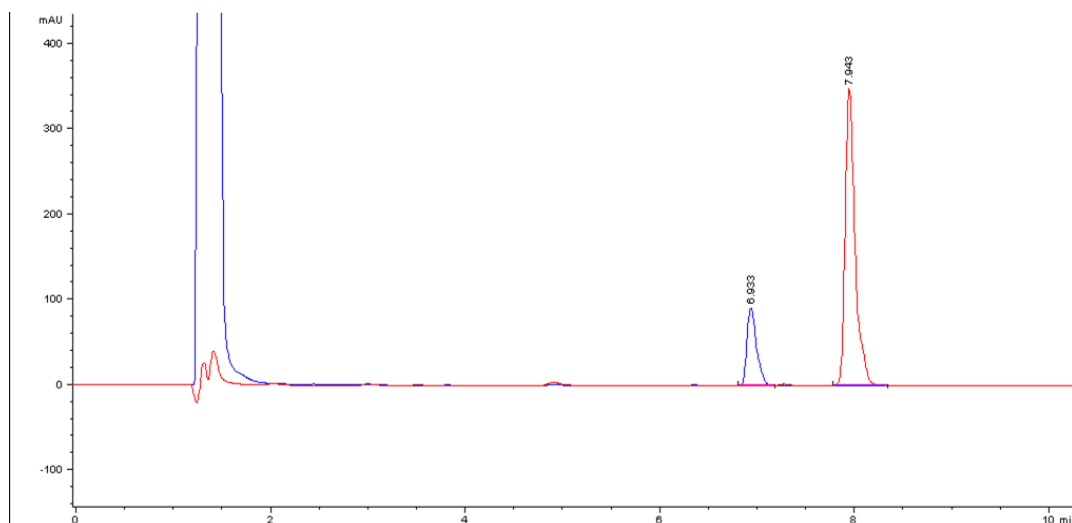
**Tip for analysis:** For ubiquitous fatty acids, such as stearic acid (present in soap, hand creams, and cosmetics), very low-level MS detection can be challenging because of the presence of such acids in the lab environment. We encourage checking for the presence of such analytes in blanks and double blanks.

## Appendix: Typical Kit Performance Data (CellMosaic)

### Labeling Efficiency Quantitation

Labeling efficiency is assessed using Fmoc-Cys(Trt)-OH and quantitated by UV/HPLC analysis. **Figure 1** shows a typical HPLC profile of Fmoc-Cys(Trt)-OH and LQA-labeled product. **Table 1** shows the labeling efficiency for various amounts of carboxylic acid. The labeling is almost quantitative for < 200 nmol.

**Figure 1:** A typical C4-HPLC profile of Fmoc-Cys(Trt)-OH (Red, 5 nmol) and LQA-labeled product (blue, 0.25 nmol)

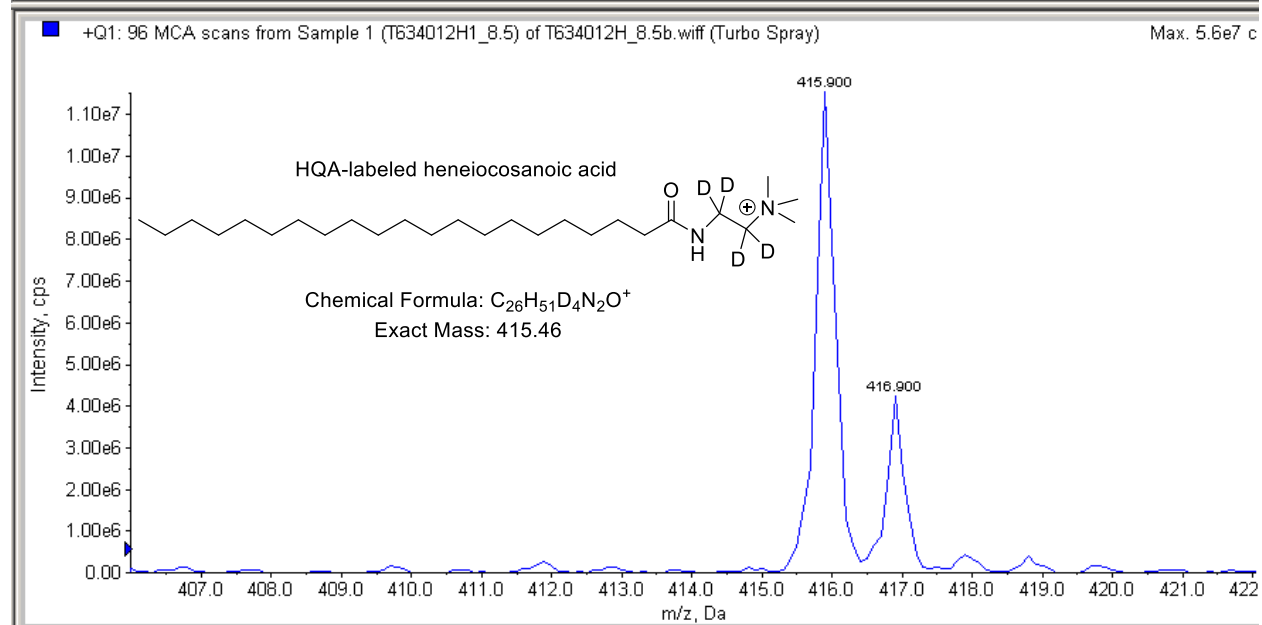
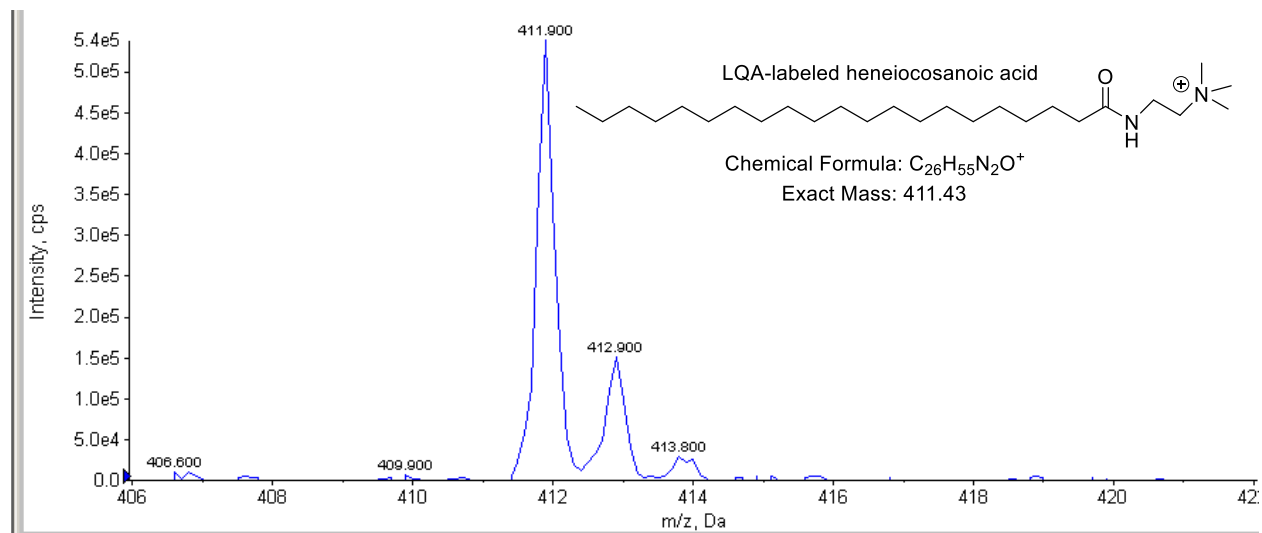


**Table 1:** Labeling efficiency of Fmoc-Cys(Trt)-OH (quantitation by C4 HPLC at 280 nm) following the standard protocol.

	Fmoc-Cys(Trt)-OH (nmol)	Labeling efficiency
<b>LQA (240 nmol)</b>	2.5	>99%
	50	>99%
	200	>70%
<b>HQA (240 nmol)</b>	2.5	>99%
	50	>90%
	200	>80%

## MS analysis of fatty acids after labeling

### Example 1: MS analysis of LQA- and HQA-labeled heneicosanoic acid



**Example 2: MS analysis of LQA- and HQA-labeled 15-methylpalmitic acid**

