

Sherlock[™] Chromatographic Analysis System (CAS) Fatty Acid Analysis & Microbial Identification

Specification Sheet

General Description

The Sherlock[™] Chromatographic Analysis System (CAS) automatically identifies fatty acid methyl esters (FAMEs) by gas chromatography. The FAME profile can be used for fatty acid compound identification (e.g. marine oil analysis, PLFA analysis, etc.), or the entire fatty acid profile can be compared by pattern recognition to one of Sherlock's microbial libraries (e.g. environmental aerobic bacteria).

The Sherlock chromatographic analysis software integrates with the Shimadzu Corporation LabSolutions™ software to automate analyses using the GC-2010/2030 instrument." Sherlock's pattern recognition algorithms, combined with its calibration mixture, standardize each Shimadzu GC, so that results are objective and reproducible across laboratories.

Libraries & Methods

The primary Sherlock methods and libraries available include:

Fatty Acid Compound Naming and Quantitation*

- Microbial Community Analysis (PLFA)
 150 soil phospholipid fatty acids
- Marine Oil Analysis (MARINE)
 - 147 marine fatty acids, including Omega-3s, Omega-6s and Omega-9s

Microbial Libraries

Environmental Aerobes (TSBA)

Environmental microbiology, Plant protection & Pharmaceutical QC

- 916 species entries
- Culture Media: TSBA agar
- Incubation Temp: 28°C ± 2°C
- Incubation Time: 24 ± 2 hours

Biothreat Agents (BTR)

Biodefense, Public health

- 37 species entries, including 6 major bacterial threat agents
- Culture Media: Blood agar
- Incubation Temp: 35°C ± 2°C
- Incubation Time: 24 ± 2 hours

Anaerobes (MOORE & BHIBLA)

Anaerobic microbiology

- Plate-grown: 156 entries
- Broth grown: 769 entries
- Culture Media: varies
- Incubation Conditions: varies

Environmental Yeast (YST28)

Environmental microbiology, Plant pathology & Soil science

- 196 species entries
- Culture Media: SDA agar
- Incubation Temp: 28°C ± 2°C
- Incubation Time: 24 ± 2 hours

***Note:** Remaining sections on this page apply to microbial ID only. Note, Quantitation requires the use of an internal standard.

Low Costs Per Sample

It costs less than \$3.00 USD per sample for all consumables, including reagents, gases, calibration standards, glassware, and culture media.

Instrument Throughput

Following the sample preparation, sample vials are loaded into the 2010/2030 GC sample tray. The Sherlock CAS automatically takes over and analyzes each sample.

- Rapid Methods (Aerobes & Biothreat agents) process <u>6 samples/hour</u>
- Sensitive Methods (Anaerobes Yeast) process <u>2 samples/hour</u>

Culturing

For microbial identification, the Sherlock CAS requires pure cultures. With standard laboratory techniques, a single subculture from the primary isolation plate, incubated for 24 hours, is typically sufficient for performing the analysis. Slow-growing organisms and anaerobes typically require 48-hour incubation times.

Sample Preparation

With inexpensive reagents, available from almost any chemical supply house, a technician averages <u>5 minutes</u> per sample to prepare a batch of 30 samples. Each sample is prepared for analysis using a liquid-liquid extraction in a single test tube.

- Harvesting a small quantity (~20mg cells) from the culture plate is the most labor-intensive step. It will typically take 1 hour or less to harvest cells from 30 plates into 30 test tubes.
- The four-step liquid-liquid extraction process requires about 1½ hours or less for a batch of 30 samples. During the extraction process, there are approximately 35 minutes of "wait time" available for the technician to do paper work and other tasks.
- The sample preparation is the same, regardless of microbial type. It is <u>not</u> necessary to do a Gram stain or other offline tests before preparing and analyzing a sample.

Bio-Safety

The first step of the extraction procedure treats the cells with a sodium hydroxide solution for 30 minutes in a 100°C water bath, which kills the microbe.

Hardware

The Sherlock CAS is composed of a Windows[®]-based computer loaded with the MIDI Sherlock and Shimadzu Corporation LabSolutions software. The computer is interfaced to MIDI-configured Shimadzu GC:

Shimadzu GC-2010/2030

- Inlet: Split/splitless
- Detector: FID
- Column: J&W Ultra 2, 25m x 0.2mm x 0.33µm film thickness
- **Tray:** standard 12 vial tray or optional 150 vial tray
- Syringe: 10µm, fixed needle
- Liner: Split/splitless focus type
- Dimensions & Weight: Depend on GC model and tray size

Gases

The Sherlock requires a specific type and quality of gases to function properly:

Carrier Gas

- Hydrogen, 99.999%+, 150 cc/min+
- Note: Helium cannot be used

Makeup Gas

- Nitrogen, 99.999%+
- Industrial Grade Air, < 1ppm THC

Analysis Software

This software enables a user to explore relationships between sample data using:

- Dendrogram plots
- Histograms
- Principal component analysis (PCA) with 2-D plots

The graphics can be exported to Microsoft Office[®] and other packages for further analysis and for research publications.



Data Export Software

This software enables a user to export sample data, fatty acid profiles, library match results and other information to Excel[®] spreadsheets and Access[®] databases. Uses for *Data Export* include:

- Custom FAME or PLFA analysis
- Summary reports for sample sets
- Research and publications
- Data mining
- Trend analysis

Library Generation Software

This software enables a user to create custom libraries from any sample data. Uses for *Library Generation* include:

- QC of proprietary strains
- Add species that are not in the standard libraries
- Alternative growth conditions
- Non-microbial sources

PLFA Analysis Tools

This analysis software is packaged with the PLFA & MARINE Methods and enable users to automatically perform complex calculations that are relevant to microbial community analysis or marine oil analysis. These include some of the following:

- Adjusting for the molarity of different fatty acid compounds
- Scaling by a known amount of internal standard (e.g. a 19:0 saturated phospholipid)
- Categorizing results based on Fatty Acid types (e.g. by omega position, such as Omega-3s vs Omega-6s)
- Calculating microbial ratios (e.g. biomass of Fungi:Bacteria)
- Calculating concentrations of key fatty acids (e.g. the mg. of DHA and EPA in fish oil capsules).

Markets Using Sherlock

- ✓ Biological Sciences (ID)
- ✓ Bioenergy (FAME, PLFA & ID)
- Bioremediation (PLFA)
- ✓ Crop Science (PLFA & ID)
- ✓ Environmental Science (PLFA & ID)
- ✓ Food Science (FAME & ID)
- ✓ Marine Science (FAME & ID)
- ✓ Pharmaceutical QC (ID)
- ✓ Soil Science (PLFA & ID)
- ✓ Supplement Industry (FAME & ID)
- ✓ Taxonomy Studies (FAME & ID)



Sherlock™ Chromatographic Analysis System with GC-2010 *Plus*

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