



Sherlock™ Chromatographic Analysis System

Microbial Community Analysis (PLFA) & 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. soil microbial community analysis), or the entire profile can be compared to one of Sherlock's microbial libraries (e.g. environmental aerobic bacteria).

The Sherlock chromatography analysis software is combined with a Shimadzu Corporation GC-2010 Plus instrument and LabSolutions™ software. Sherlock's pattern recognition algorithms, combined with its calibration mixture, standardize each Shimadzu GC, so that the results are reproducible across laboratories.

Libraries & Methods

The primary Sherlock methods and libraries available include:

Compound Naming Only

Phospholipid Fatty Acids (PLFA)*

- Soil microbial community analysis
- 165 fatty acids and related compounds
- Culture Media: not applicable
- Incubation Conditions: not applicable
- Used with any PLFA extraction method

Compound Naming & Libraries

Environmental Aerobes (TSBA)

- Environmental microbiology & Plant pathology
- 916 species entries
- Culture Media: TSBA agar
- Incubation Temp: 28°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
- 196 species entries
- Culture Media: SDA agar
- Incubation Temp: 28°C ± 2°C
- Incubation Time: 24 ± 2 hours

**Note: The PLFA extraction & method are covered in a separate document. The remaining sections on this page apply to microbial identification from pure cultures only.*

Low Costs Per Sample

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

Instrument Throughput

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

- **Rapid** methods (Aerobes only) process 6 samples per hour
- **Sensitive** methods (Anaerobes and Yeast) process 2 samples per hour

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 5 minutes 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 not 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 Plus

- **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:** 52cm x 53cm x 44cm (L x W x H)
- **Weight:** 30kg

Gases

The Sherlock requires a specific type and quality of gases in order 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
- Neighbor-joining trees
- Principal component analysis (PCA) with 2-D plots and histograms

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



Veteran-Owned Small Business

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. There are many applications for custom reports and calculations created using Excel, Access, and other data analysis tools:

- Custom 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:

- Quality control of proprietary strains used in production processes
- Assign an identity to organisms that do not have a published taxonomy
- Alternative growth conditions
- Research

PLFA Analysis Tools

This analysis software is packaged with the PLFA Methods and enables a user to automatically perform complex calculations that are relevant to microbial community analysis. These include the following:

- Adjusting for the molarity of different fatty acid compounds
- Scaling by a known amount of internal standard (e.g. a saturated 19:0 phospholipid)
- Categorizing results based on Fatty Acid types (e.g. iso/anteiso, omega position)
- Categorizing results based on microbial types (e. g. Gram-positive bacteria, fungi)
- Calculating microbial ratios (e.g. Fungi/Bacteria)
- Calculating the biomass in nmol PLFA g⁻¹

Markets Using Sherlock

- ✓ Agricultural biotechnology (PLFA)
- ✓ Bioremediation (PLFA)
- ✓ Crop Science (PLFA & ID)
- ✓ Environmental Science (PLFA & ID)
- ✓ Food Science (PLFA & ID)
- ✓ Marine Science (ID)
- ✓ Microbial Culture Collections (ID)
- ✓ Public Health (ID)
- ✓ Renewable Energy (PLFA)
- ✓ Soil Science (PLFA)
- ✓ Taxonomy Studies (ID)



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MIDI, Inc.
125 Sandy Drive
Newark, Delaware 19713 USA
Phone: 302-737-4297
Fax: 302-737-7781
Email: sales@midi-inc.com
Web: www.midi-inc.com

