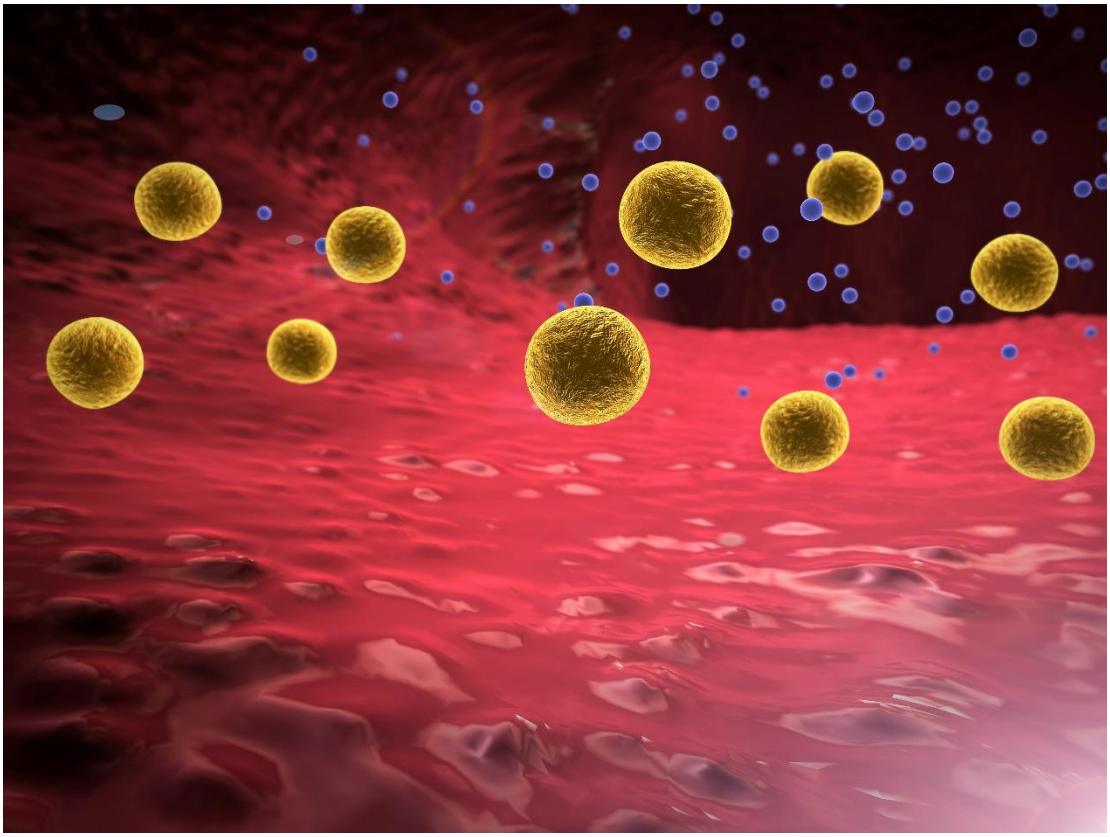
BILE ACID/CARNITINE/STEROL METABOLITE LIBRARY OF STANDARDS

Cat No. BACSMLS



CONVENIENT 96-WELL FORMAT Easy storage, no glass bottles

Plated to allow row multiplexing for efficient processing

HIGH PURITY and STABLE

Supplied as 5 µg dried weight; plenty of material for multiple injections

MLSDiscovery™ SOFTWARE

Data processing, data collection and data reduction tool creates libraries in hours

IROA TECHNOLOGIES

...making metabolic profiling easy®

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BACSMLS™ (Bile Acid/ Carnitine/ Sterol Metabolite Library of Standards) is a collection of high-quality bile acids, carnitines and sterols. These are high purity (>95%) compounds supplied in an economical, ready-to-use format. The library of standards is most commonly used to provide retention times and spectra for these key metabolic compounds, help optimize mass spectrometry analytical protocols, and qualify and quantify mass spectrometry sensitivity and limit of detection. These compounds are involved in the regulation of various metabolic processes and are of great interest in microbiome research and studies involving lipid and energy homeostasis.

BACSMLS comes with **MLSDiscovery™**, a software tool to support the extraction, manipulation, and storage of the data generated when using the BACSMLS Library of authentic metabolite standards.

Features and Benefits

Compounds

BACSMLS contains 96 unique small molecule primary metabolites and intermediates covering key metabolic pathways, including the following classes of compounds:

- Bile acids
- Carnitines
- Sterols

Convenient

- High purity metabolites, pre-weighed, supplied dried, 5µg each compound
- Ideal for mass spectrometry metabolomics applications

Formatted

BACSMLS contains 96 unique small molecule metabolites.

- Arrayed in a 96-well plate
    - Greiner MASTERBLOCK® #780215, polypropylene deep-well (total volume per well = 1. 2mL) in combination with seal, Greiner VIEWseal #676070
- Suitable for manual and automated work flow

Software

MLSDiscovery software package is distributed with and is tailored to work with IROA Metabolite Standards to help build a physical mass spectral library using the analytical conditions that are normally employed in the user laboratory. MLSDiscovery collects spectra, retention time, mass and relative intensity information for compounds, fragments and adducts.

The software supports most MS data files. The requirements of the program are that:

1) The computer should have at least 8 GB of RAM; Windows 7 or higher;
2) Data files must be able to be converted to mzXML format.

To facilitate the use of the program the MLSDiscovery User Manual is provided to help you run through the standard workflow.

Plate Map

The library compounds are arranged in (1) one 96-well polypropylene rack with alphanumeric assigned positions. Please refer to plate map excel sheet that comes with the library for product locations and identifiers.

Occasionally the map plate will change due to the availability of compounds. Although we try to make sure that the compounds of each row have distinct molecular weights and can be multiplexed, users should refer to the plate map before proceeding.

The plate map contains descriptors and represents information gathered from multiple databases and therefore may contain errors. We suggest that the information provided is carefully reviewed. To help build a better database, please report any discrepancies.

The excel spreadsheet plate map includes columns **A-R** as follows:

A. Plate number - total number of plates is one (1).
B. Plate row letter - rows are marked A-H.
C. Plate column number - columns are numbered 1-12.
D. Primary compound name – from KEGG or PubChem where available.
E. SMILES – from https://pubchem.ncbi.nlm.nih.gov/search/help_search.html#Smiles

SMILES -- **S**implified **M**olecular **I**nput **L**ine **E**ntry **S**ystem, a chemical structure *line notation* (a typographical method using printable characters) for entering and representing molecules. SMILES strings can be imported or exported from many molecular editors.

F. **Molecular formula** – formula of neutral form without salts or water (except in cases where compound has an innate positive charge).
G. **KEGG ID or ChemSpider ID (CSID)** – KEGG number where available, otherwise CSID provided.
H. **CAS ID**– a unique numerical identifier assigned by Chemical Abstracts Service, a division of the American Chemical Society, to every chemical substance described in the open scientific literature.
I. **HMDB/YMDB ID** – Human Metabolome Data Base or Yeast Metabolome Data Base ID number. Either the chemical name or the CAS number was used to search for the HMDB/YMDB ID entry.
J. **Neutral monoisotopic mass**
K. **METLIN ID** – Scripps Center for Metabolomics and Mass Spectrometry; HMDB, KEGG or CAS number used for METLIN lookup.
L. **PC CID** – PubChem Compound Database ID; HMDB, KEGG or CAS number used for PC CID lookup.
M. **PC SID** – PubChem Substance Database ID; PC CID, KEGG or CAS number used for PC SID lookup.
N. **CHEBI** – Chemical Entities of Biological Interest (ChEBI); HMDB, KEGG or CAS number used for ChEBI lookup.
O. **Supplier Cat. No.**
P. **Supplier compound name**
Q. **Supplier CAS ID**
R. **Supplier URL**

Note: Although some of the compounds as seen in the plate wells will be colorless, many dried compounds naturally exhibit color.

Preparation Instructions

Spin down the contents of each 96-well plate in a 96-well plate holder centrifuge, 3000rpm, for 2-3 minutes, to ensure any loose material is deposited at the bottom of each well.

BACSMLS compounds are conveniently provided at 5 µg per well, enough for multiple injections. The compounds of the BACSMLS can either be used as standards and injected individually or mixed in such a way that the entire library may be examined with reasonable efficiency. Across all plates the compounds in each row *may* all have unique masses; mixing compounds by row *may* allow multiple compounds to be analyzed per injection.

The following are suggestions and dependent on user chromatography and instrumentation.

1) Individual injections. As standards, each well represents a single compound; thus the entire library may be examined in great detail in 96 injections for each of the unique compounds. (Volumes of approximately 250 µL may be considered).
2) Simple multiplex injections. If each row of each plate is pooled, then the entire collection may be analyzed in 8 injections of simple mixtures. Keep the well volume to 100 µl or less to prevent loss due to dilution and take 5-10 µl of each well for the pooled sample, then inject 2, 4, or 6 µl of the pooled material as needed.
   Note: Be sure to check the individual masses across plate rows to ensure these compounds can be separated with the chromatographic system employed.

Solubilization and compound preparation procedure

The following are suggestions and dependent on user chromatography and instrumentation.

1) Most compounds can be solubilized using high-quality methanol. (Exceptions are: A1, B8, B11, B12, C6, C12 – solubilize in high-quality chloroform and B10 – solubilize in high-quality chloroform: Methanol (1:1). Pipet liquid up and down in the well 2-3 times to facilitate solubilization.
2) Pool compounds for multiplexing. Again, be sure to check the plate map to ensure you can adequately separate the compounds using your chromatographic system prior to pooling.

Precautions and Disclaimer

The BACSMLS product is for laboratory research use only. Wear safety glasses and handle with gloves. Avoid contact with skin and eyes. Please consult the Safety Data Sheet for safe handling practices and hazards information.

Storage/Stability

Store plates at -20° C. Once diluted the plates should be resealed and kept at -20° C or -80° C for long-term storage and protected from moisture and light. Avoid repeated freeze/thaw cycles.

Metabolite Libraries Available from IROA

MS Metabolite Library of Standards (MSMLS) - 600 unique compounds arrayed in seven (7) 96-well plates that span a broad range of primary metabolism; 5 µg per well.

Large Scale Metabolite Library of Standards (LSMLS) - 600 unique compounds arrayed in seven (7) 96-well plates that span a broad range of primary metabolism; 1 mg per well.

Bile Acid Carnitine Sterol Metabolite Library of Standards (BACSMLS) - 96 bile acid, carnitine and sterol metabolites covering key metabolic pathways; 5 µg per well.

Fatty Acid Metabolite Library of Standards (FAMLS) - 96 unique small molecule fatty acid metabolites covering key metabolic pathways; 5 µg per well.

Organic Acid Metabolite Library of Standards (OAMLS) - 96 unique small molecule organic acid metabolites covering key metabolic pathways; 5 µg per well.

Amino Acid/Peptide Metabolite Library of Standards (AAPMLS) – 96 unique metabolites including acetylated, methylated and hydroxy amino acids and dipeptides which are building blocks of proteins in many prokaryotic and eukaryotic organisms; 5 µg per well.

Microbiome Metabolite Library of Standards (GUTMLS) – 185 unique small biochemicals that the gut microbiome produces and interacts with including bacterial, dietary and host xenobiotic metabolites; 5 µg per well.

Phytochemical Metabolite Library of Standards (PHYTOMLS) - 364 unique primary and secondary plant metabolites obtained from consuming diets containing fruits, vegetables, whole grains, legumes, nuts and plant-based beverages; 5 µg per well.

Polyphenol Metabolite Library of Standards (PPMLS) – 80 unique high-quality small biochemicals that have a polyphenol structure (i.e., several hydroxyl groups on aromatic rings). Polyphenols are found in many plant foods including fruits, vegetables, cereals, dry legumes, chocolate, oils, spices, and some beverages, including red wine.

Legal Information

Acknowledgements

We gratefully acknowledge usage of the following websites and databases for their publicly accessible information.

Database	Website

The Human Metabolome Database (HMDB), v 2.5 [1-3]	http://www.hmdb.ca/
The Yeast Metabolome Database [4]	http://www.ymdb.ca/
Chemical Entities of Biological Interest (ChEBI) [5]	https://www.ebi.ac.uk/chebi/
Chemical Abstracts Service (CAS) REGISTRY Database [6]	https://www.cas.org/
Kyoto Encyclopedia of Genes and Genomes (KEGG) [7]	http://www.genome.jp/kegg/
The METLIN Metabolomics Database [8-9]	http://metlin.scripps.edu/index.php
The PubChem Compound and Substance Database [10]	https://pubchem.ncbi.nlm.nih.gov/

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