LARGE SCALE Metabolite Library of Standards

Cat No. LSMLS

CONVENIENT 96-WELL FORMAT Easy storage, no glass bottles

Plated to allow row multiplexing for efficient processing

HIGH PURITY and STABLE

Supplied as 1 mg dried weight; plenty of material for multiple injections

MLSDiscovery™ SOFTWARE

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

info@iroatech.com www.iroatech.com 734-265-0884

LSMLS™ (Large Scale Metabolite Library of Standards) is a collection of high quality small biochemical molecules that span a broad range of primary metabolism. 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 key metabolic compounds, help optimize mass spectrometry analytical protocols, qualify and quantify mass spectrometry sensitivity and limit of detection, and for functional cellular assays and phenotypic screening.

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

Features and Benefits

Compounds

LSMLS contains 600 unique small molecule metabolites

Broad metabolite spectrum, key primary metabolites and intermediates covering key metabolic pathways, including the following classes of compounds:

- Carboxylic acids, amino acids
- Biogenic amines, polyamines
- Nucleotides, coenzymes and vitamins
- Mono- and disaccharides
- Fatty acids, lipids, steroids, and hormones

Convenient

- High purity metabolites, pre-weighed, supplied dried, 1 mg each compound
- Ideal for mass spectrometry metabolomics applications

Formatted

LSMLs contains a total of 600 compounds.

- Arrayed in 96-well format
- 7 polypropylene racks

IROA TECHNOLOGIES
...making metabolic profiling easy®

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- Greiner MASTERBLOCK® #780215, polypropylene deep-well (total volume per well = 1. 2mL) in combination with Agilent 24214-001 Peelable PlateLoc Heatseals
- Suitable for manual and automated workflow

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 (7) seven 96-well polypropylene racks with alphanumeric assigned positions. Please refer to the plate map excel sheet that comes with the library for product locations and identifiers.

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 identifiers as follows:

Plate number - total number of plates is seven, 1-7.
Plate row letter - rows are marked A-H.
Plate column number - columns are numbered 1-12.
Primary compound name – from KEGG or PubChem where available.
Molecular formula – formula of neutral form without salts or water (except in cases where compound has an innate positive charge).

CAS – 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.
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.

KEGG ID – KEGG number where available.
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.
Neutral monoisotopic mass
METLIN ID – Scripps Center for Metabolomics and Mass Spectrometry; HMDB, KEGG or CAS number used for METLIN lookup.
PC CID – PubChem Compound Database ID; HMDB, KEGG or CAS number used for PC CID lookup.
PC SID – PubChem Substance Database ID; PC CID, KEGG or CAS number used for PC SID lookup.
CHEBI – Chemical Entities of Biological Interest (ChEBI); HMDB, KEGG or CAS number used for ChEBI lookup.
Inchikey – a 27-character long identifier based on The IUPAC International Chemical Identifier (InChI™).
Supplier Cat No.
Supplier product name
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.

LSMLS compounds are conveniently provided at 1 mg per well, enough for multiple injections. The compounds of the LSMLS 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.

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, due to compound availability this is not always possible, and users should refer to the plate map before proceeding.

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

1) Individual injections. As standards, each well represents a single compound; the entire library may be examined in great detail in 600 injections for each of the unique compounds. (Volumes of 100-300 µL may be considered).
2) Simple multiplex injections. If each row of each plate is pooled, then the entire collection may be analyzed in 51 injections of simple mixtures. Keep the well volume to 200 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) Plates 1-5: Add 5% of final volume (up to 20 µl) of very clean MeOH to every well of every plate. Add high purity water to make up the desired volume. The addition of water ensures the solubilization of the more polar compounds. We suggest a final 5% methanol solution. Pipet liquid up and down in the well 2-3 times to facilitate solubilization.
2) Plate 6: Add 10% of final volume of high purity water, mix, and then add 100% Ethanol to make up the final volume.
3) Plate 7: These wells contain mostly lipid-like compounds. We recommend solubilizing Row A in 100% Chloroform, Row B in 90% Methanol (add 10% of final volume of high purity water, mix, and then add 100% Methanol to make up the final volume), and for Rows C-E add 20% of final volume of high purity DMSO, mix, and then add 100% Methanol to make up the final volume.

4) 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 LSMLS 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 aliquoted, 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

Mass Spectrometry 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 to have a direct influence on the composition and signaling pathways of the gut microbiota.

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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