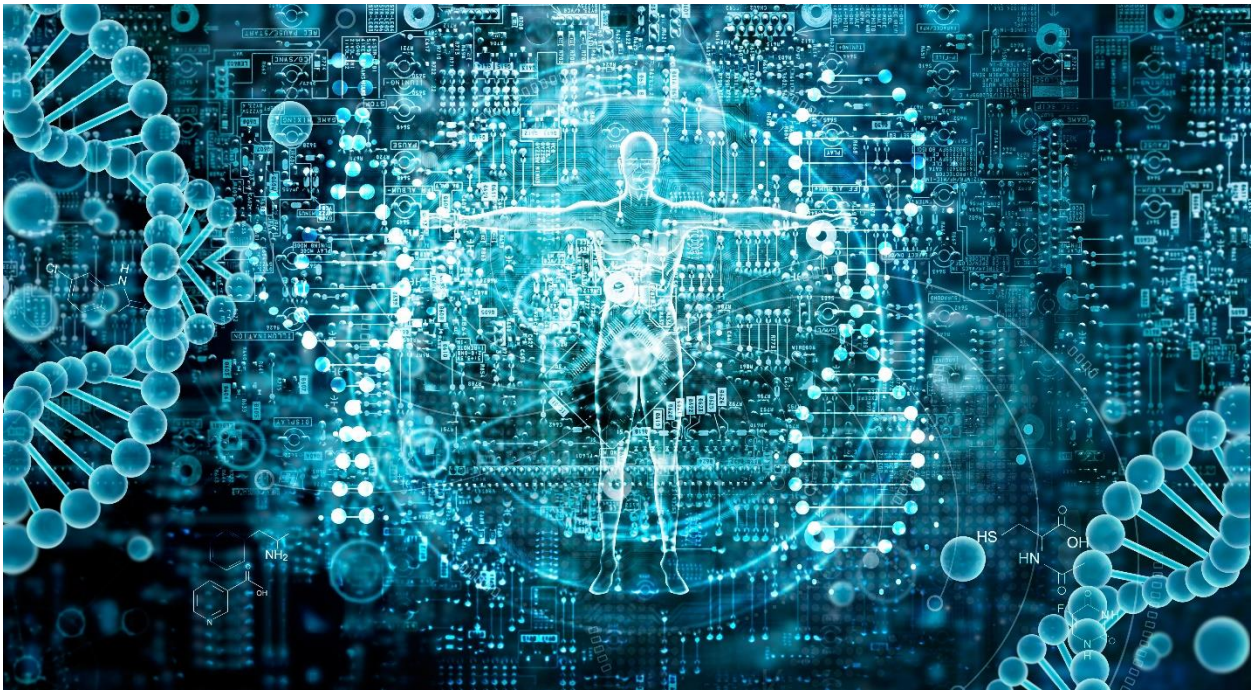
MASS SPECTROMETRY METABOLITE LIBRARY OF STANDARDS

Cat No. MSMLS



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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MSMLS™ (Mass Spectrometry 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, and qualify and quantify mass spectrometry sensitivity and limit of detection.

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

Features and Benefits

Compounds

MSMLS contains over 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, 5µg each compound
- Ideal for mass spectrometry metabolomics applications

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MSMLS contains over 600 unique small molecule metabolites, a total of 634 compounds.

- Arrayed in 96-well format
- 7 polypropylene racks
    - Greiner MASTERBLOCK® #780215, polypropylene deep-well (total volume per well = 1. 2mL) in combination with seals, 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 a 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 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-Q** as follows:

A. Plate number - total number of plates is seven, 1-7.
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.

Note: The formulae and SMILES for nucleotides are represented as neutral forms (N+ and O-).

F. **Molecular formula** – formula of neutral form without salts (except in cases where compound has an innate positive charge).
G. **KEGG ID** – KEGG number where available.
H. **CAS IS** – 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 URL**

Preparation Instructions

MSMLS compounds are conveniently provided at 5 µg per well, enough for multiple injections. The compounds of the MSMLS 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.

Note: Plate 6 contains sugar H2O soluble compounds of which have masses too close in range to inject together.

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

1) Individual injections. Each well represents a single compound; the entire library may be examined in great detail in 603 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 56 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) Plates 1-5: We recommend adding methanol to begin solubilization and then diluting as required with water. We suggest adding 5 µL of MeOH, vortex a couple minutes, then add 95 µL of [95/5 water/MeOH] = 50 µg/mL of each metabolite in each well, vortex again. Multiplex the samples in each row and inject 5 µL to the LC/MS for both Pos and Neg acquisitions. Good results should be achieved in each mode.
2) Plate 6: These wells contain compounds that are soluble in 40% Ethanol or Methanol (except for the sugar H2O soluble compounds in plate 6).
3) Plate 7: These wells contain mostly lipid-like compounds. We recommend solubilizing these compounds in 1:1 chloroform : methanol. If you are not comfortable injecting the multiplexed sample of CHCl3/MeOH on a reverse phase system without adding any aqueous solvent, start with about 15-20 µL of ACN/IPA or ACN/EtOH, vortex 5 min, then add 15-20 µL of aqueous starting mobile phase, and give a final vortex 5 min before injections. If the mobile phase has acid in it then just use the mobile phase minus the acid.

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

Mass Spectrometry Metabolite Library of Standards (MSMLS) - Over 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) - Over 500 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.

Precautions and Disclaimer

The MSMLS 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.

Legal Information

Acknowledgements

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

IROA TECHNOLOGIES
...making metabolic profiling easy®

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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/

References

[1] Wishart DS, Tzur D, Knox C, et al., HMDB: the Human Metabolome Database. Nucleic Acids Res. 2007 Jan;35 (Database issue):D521-6. 17202168.

[2] Wishart DS, Knox C, Guo AC, et al., HMDB: a knowledgebase for the human metabolome. Nucleic Acids Res. 2009 37(Database issue):D603-610. 18953024.

[3] Wishart DS, Jewison T, Guo AC, Wilson M, Knox C, et al., HMDB 3.0 — The Human Metabolome Database in 2013. Nucleic Acids Res. 2013. Jan 1;41(D1):D801-7. 23161693.

[4] Jewison T, Neveu V, Lee J, Knox C, Liu P, Mandal R, Murthy RK, Sinelnikov I, Guo AC, Wilson M, Djoumbou Y and Wishart DS. YMDB: The Yeast Metabolome Database. Nucleic Acids Res. 2012 Jan; 40(Database Issue): D815-20 PubMed: 22064855.

[5] Hastings J, de Matos P, Dekker A, Ennis M, Harsha B, Kale N, Muthukrishnan V, Owen G, Turner S, Williams M, Steinbeck C. The ChEBI reference database and ontology for biologically relevant chemistry: enhancements for 2013. Nucleic Acids Res. 2013. Jan;41(Database issue):D456-63. doi: 10.1093/nar/gks1146. Epub 2012 Nov 24.

[6] CAS REGISTRY, Division of the American Chemical Society

[7] Kanehisa M, Goto S. "KEGG: Kyoto Encyclopedia of Genes and Genomes". Nucleic Acids Res. 2000 28 (1): 27–30. doi:10.1093/nar/28.1.27. PMC 102409.PMID 10592173.

[8] Tautenhahn R, Cho K, Uritboonthai W, Zhu Z, Patti G, Siuzdak G. An accelerated workflow for untargeted metabolomics using the METLIN database. Nature Biotechnology 2012 30: 826–828. doi:10.1038/nbt.2348.

[9] Smith CA, I'Maille G, Want EJ, Qin C, Trauger SA, Brandon TR, Custodio DE, Abagyan R, Siuzdak G. METLIN: a metabolite mass spectral database. The Drug Monit 2005 27 (6): 747–51. doi:10.1097/01.ftd.0000179845.53213.39. PMID 16404815.

[10] Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, Han L, He J, He S, Shoemaker BA, Wang J, Yu B, Zhang J, Bryant SH. PubChem Substance and Compound databases. Nucleic Acids Res. 2016 Jan 4; 44(D1):D1202-13. Epub 2015 Sep 22 [PubMed PMID: 26400175] doi: 10.1093/nar/gkv951 [Free Full Text at Oxford Journals].

IROA MLS Referenced Peer-Reviewed Publications

Fraisier-Vannier O, Chervin J, Cabanac G, et al. MS-CleanR: A Feature-Filtering Workflow for Untargeted LC-MS Based Metabolomics. *Anal Chem*. 2020;92(14):9971-9981. doi:10.1021/acs.analchem.0c01594. PMID: 32589017

Misra BB, Olivier M. High Resolution GC-Orbitrap-MS Metabolomics Using Both Electron Ionization and Chemical Ionization for Analysis of Human Plasma [published online ahead of print, 2020 Feb 10]. J Proteome Res. 2020;10. doi:10.1021/acs.jproteome.9b00774. PMID: 31978300

Vargas F, Weldon KC, Sikora N, et al. Protocol for Community-created Public MS/MS Reference Spectra Within the Global Natural Products Social Molecular Networking Infrastructure [published online ahead of print, 2020 Jan 13]. Rapid Commun Mass Spectrom. 2020; e8725. doi:10.1002/rcm.8725. PMID: 31930757

Nye LC, Williams JP, Munjoma NC, et al. A comparison of collision cross section values obtained via travelling wave ion mobility-mass spectrometry and ultra high performance liquid chromatography-ion mobility-mass spectrometry: Application to the characterisation of metabolites in rat urine. J Chromatogr A. 2019 Sept ;1602:386–396. doi:10.1016/j.chroma.2019.06.056. PMID: 31285057

Dueñas ME, Larson EA and LEee YJ. Toward Mass Spectrometry Imaging in the Metabolomics Scale: Increasing Metabolic Coverage Through Multiple On-Tissue Chemical Modifications. Front. Plant Sci. 2019 July 10;860. Doi 10.3389/fpls.2019.00860. PMID: 31354754

Pezzatti J, González-Ruiz V, Codesido S, Gagnebin Y, Joshi A, Guillarme D, Schappler J, Picard D, Boccard J, Rudaz S. A scoring approach for multi-platform acquisition in metabolomics. J Chromatogr A. 2019 May 10;1592:47-54. doi: 10.1016/j.chroma.2019.01.023. Epub 2019 Jan 10. PMID: 30685186

González-Ruiz V, Schvartz D, Sandström J, Pezzatti J, Jeanneret F, Tonoli D, Boccard J, Monnet-Tschudi F, Sanchez JC, Rudaz S. An Integrative Multi-Omics Workflow to Address Multifactorial Toxicology Experiments. Metabolites. 2019 Apr 24;9(4). pii: E79. doi: 10.3390/metabo9040079. PMID: 31022902

Nichols CM, Dodds JN, Rose BS, et al. Untargeted Molecular Discovery in Primary Metabolism: Collision Cross Section as a Molecular Descriptor in Ion Mobility-Mass Spectrometry. Analytical Chemistry. 2018 Dec;90(24):14484-14492. DOI: 10.1021/acs.analchem.8b04322. PMID: 30449086

Pimentel G, Burton KJ, von Ah U, Bütikofer U, Pralong FP, Vionnet N, Portmann R, Vergères G. Metabolic Footprinting of Fermented Milk Consumption in Serum of Healthy Men. J Nutr. 2018 Jun 1;148(6):851-860. doi: 10.1093/jn/nxy053. PMID: 29788433

Thomason K, Babar MA, Erickson JE, Mulvaney M, Beecher C, MacDonald G. Comparative physiological and metabolomics analysis of wheat (*Triticum aestivum* L.) following post-anthesis heat stress. PLoS ONE 2018 June13(6): e0197919. https://doi.org/10.1371/journal.pone.0197919.

Nemkov T, Hansen KC, D'Alessandro A. A three-minute method for high-throughput quantitative metabolomics and quantitative tracing experiments of central carbon and nitrogen pathways. Rapid Commun Mass Spectrom. 2017 Apr 30;31(8):663-673. doi: 10.1002/rcm.7834. PMID: 28195377

Depke T, Franke R, Brönstrup M. Clustering of MS^2 spectra using unsupervised methods to aid the identification of secondary metabolites from Pseudomonas aeruginosa. J Chromatogr B Analyt Technol Biomed Life Sci. 2017 Dec 15;1071:19-28. doi: 10.1016/j.jchromb.2017.06.002. Epub 2017 Jun 4. PMID: 28642031

González-Ruiz V, Pezzatti J, Roux A, Stoppini L, Boccard J, Rudaz S. Unravelling the effects of multiple experimental factors in metabolomics, analysis of human neural cells with hydrophilic interaction liquid chromatography hyphenated to high resolution mass spectrometry. J Chromatogr A. 2017 Dec 8;1527:53-60. doi: 10.1016/j.chroma.2017.10.055. Epub 2017 Oct 25. PMID: 29106965

Lu, X., Solmonson, A., Lodi, A. et al. The early metabolomic response of adipose tissue during acute cold exposure in mice. Sci Rep 2017 June 7**;** 3455. doi.org/10.1038/s41598-017-03108-x. PMID: 27399036

Korte AR, Stopka SA, Morris N, Razunguzwa T, Vertes A. Large-Scale Metabolite Analysis of Standards and Human Serum by Laser Desorption Ionization Mass Spectrometry from Silicon Nanopost Arrays. Anal Chem. 2016 Sep 20;88(18):8989-96. doi: 10.1021/acs.analchem.6b01186. Epub 2016 Jul 22. PMID: 27399036