MASS SPECTROMETRY METABOLITE LIBRARY OF STANDARDS FREQUENTLY ASKED QUESTIONS (FAQ)

Uses for MSMLS

Q - What is the purpose of MSMLS?

A - MSMLS provides over 600 small metabolite compounds in an easy to use format with enough material for multiple injections for the purpose of building a physical mass spectral library using the conditions that are normally employed in the user laboratory. While many mass spectral libraries are available, and can help identify compounds within a dataset, there is no substitute for generating your own library using your own parameters.

The MSMLS compounds largely represent primary metabolism and ideally suited to build libraries used to identify compounds for metabolic profiling.

The MSMLSDiscovery software provided with the kit facilitates “library building”. As each injection sample contains a known set of compounds, the software automatically processes the data for each sample and provides the extracted ion-chromatogram for the expected mass, and for each peak found collects any fragmentation data, centroids the peak and collects all adducts. The software then presents what it finds for a curation step. The curation of the complete library or “dictionary” can be completed in less than a day.

MSMLSDiscovery also provides a “sample analysis” feature that will run a targeted analysis on a centroided data file to find any compounds in the dictionary/library created during “library building”.

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Compounds

Q - Are the standards in the MSMLS isotopically labeled according to the IROA technology?

A - No, all the compounds are unlabeled (natural abundance).

Q - Is the amount of material in each well exactly 5µg?

A - 5 µL of an accurately weighed 1 mg/ml solution was placed into the well and dried down. The standard deviation of the scale used to weigh the compounds is +/- 0.1 mg

Q - Why are there duplicated compounds in the library?

A - Compounds are intentionally duplicated for QC purposes which is why the kit is defined as having over 600 "unique compounds". Many compounds placed in the well have different D/L forms, chirality or salts and have different CAS numbers.

Descriptors

Q - What is the Parent CID/ CSID?

A - This usually refers to the KEGG ID (Kyoto Encyclopedia and Genomes) number, where available.

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Q - What does “Charge” of the compound refer to?

A - Charge is only for the compounds that are by default cations or anions, such as choline. This is how it is in MS, it doesn't need to form an adduct to be detected.

Solubilization

Q - What is the recommended procedure to solubilize the compounds?

A - We recommend adding methanol to begin solubilization and then diluting as required with water. Water is necessary to ensure solubilization of the more polar compounds. For plates 6 & 7 we recommend using 1: 1: 0.3 chloroform: methanol: water as these compounds are more lipid-like (with the exception of the water-soluble sugars on plate 6).

Q - How much volume can I add to each 96 plate well?

A - Each well can hold a volume of 1.2 mL.

Chromatography

Q - What is the best chromatography to use with MSMLS?

A - There is no “best chromatography” and this is dependent upon the instrumentation available, i.e. there is no single recommended Mass Spec model and/or protocol. The library is designed to help you tailor your own system, and support your ability to build a library using authentic compounds under your source and chromatographic conditions.

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Many MSMLS users prefer a basic C-18 column run in both positive and negative mode. A HILIC or Diamond-hydride is often employed to further separate the very water soluble compounds. This increases the number of injections and the time per analysis.

Stability and Storage Conditions

Q - What is the stability and best storage conditions for the MSMLS compounds?

A - The shelf life of the kit is 2 years. Once diluted the plated should be resealed and kept at -20°C or -80°C for long-term storage, protected from moisture and light. Avoid repeated freeze/thaw cycles.

Q - What are the 96-well plates that were used to generate MSMLS.

A - Greiner MASTERBLOCK® #780215, polypropylene deep-well (total volume per well = 1. 2mL) in combination with seals, Greiner VIEWseal #676070.

Mass Spec Analysis

Q - If I switch polarity (alternating polarity) when analyzing my samples, can the MSMLSDiscovery software still be used?

A – The current MSMLSDiscovery version does not support mixed polarity data files.

Q - Can I multiplex every row in every plate?

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A - 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.

Publications

Q - Are there any publications that reference MSMLS?

A - *MetaboNews* featured and article that described the MSMLSDiscovery. A User Guide and video are provided with the kit. We expect to release an Application Note soon.

MSMLSDiscovery

Q - What operating system should I use and how much memory does the MSMLSDiscovery program require?

A Microsoft Windows 7 (64-Bit) or higher with a minimum of 8 GB memory, for improved data processing 16 GB memory is preferred. The latest version of Java should be callable.

Q - What should I do if I get a message "unable to load" when I try to download the MSMLS software?

A - This generally means that a corporate firewall is not permitting any downloads to be run. The install may need to be done by the IT department who will download and test the software.

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Q - What does it mean if I get the message "GC overhead memory limit exceeded"?

A - Java has a Garbage Collection (GC) process that tries to free up memory. This message is telling you that you are out of virtual memory. On a 64-BIT operating system you can manually change "HEAP_SIZE=8" TO "HEAP_SIZE=32".

Q - What data format is required to upload into MSMLSDiscovery

A - Convert datafiles to centroided mzXML. Most vendor files can be processed using ProteoWizard (open-sourced, cross-platform tool).

Q - What is MS depth?

A - MS depth is the number of mass spec scan levels to discover. The program supports two: 1) full scan, and 2) MS/MS.

Q - Is it possible to export the peak intensities and the area of the peaks?

A - When building the libraries, compounds definition is purely descriptive and has no quantitative aspects, thus height or area have no meaning (except in the ms/ms the user can set the percent of baseline that defines which fragments get written out). When using Discovery for sample analysis, the user can select the export type, either height or area (under Preferences: Data export).

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