

1. What are the common substance testing platforms and which one is suitable for me?

NMR: Nuclear magnetic resonance has the advantages of detecting non-damaged samples and without complicated sample pre-processing, and thus, the sample is as close to their physiological condition as possible. However it suffers from lower sensitivity, especially low abundance metabolites.

LC-MS: The combined technology of liquid chromatography and mass spectrometry has relatively high resolution, high sensitivity, and allows detection of wide range of metabolites. This is typically used to detect non-volatile polar compounds that are less than 1000 Da.

GC-MS: Gas chromatography and mass spectrometry is mainly used for identifying volatile substance or metabolites with low polarity. Typically detects molecules with lower molecular weight (< 300Da).

2. What are the criteria for screening differential metabolites?

Screening criteria for differential metabolites: select metabolites with fold change ≥ 2 and fold change ≤ 0.5 . If the difference of metabolites between the control group and the experimental group is more than 2 times or less than 0.5, the difference is considered significant. If there is biological duplication in the sample grouping, on the basis of the above, select the metabolites with $VIP \geq 1$. The VIP value represents the influence of the difference between the corresponding metabolites in the classification and discrimination of the samples in each group in the model. It is generally considered that the metabolites with $VIP \geq 1$ are significantly different.

3. Can I compare compounds from different batches? Can I compare different substances in the same sample?

Mass specs needs regular maintenance and calibration, thus quantifications from Widely-Targeted Metabolomics between different batches typically cannot be compared directly.

In the mass spec, the degree of ionization of compounds are different and thus you cannot compare different compounds from the same samples.

4. The range of substances that can be detected for metabolites?

The detection range of liquid-phase mass spectrometry should be less than 1000Da, and the molecular weight of frequently encountered macromolecular substances such as glycans, peptides, starch, pectin, and lignin are not within the detection range.

5. How to detect substances that are not in the database? Why can't I detect a substance that is in the database?

If the substance of interest is not in the database, we recommend purchasing a purified standard and we may develop a targeted assay for you.

If the substance of interest is in the database but were not detected in the samples, there maybe the following reasons:

1. The substance is not contained in samples or the content of the substance is extremely low.
2. As high-throughput detection scans thousands of compound species at a time, there may be interfering ions that prevents detection.

6. Why do you want to mix samples and how do I mix?

In order to avoid inaccurate metabolite detection differences caused by individual differences, metabolome testing recommends mixing samples, i.e. each biological sample is in principle from a mixture of at least 3 individuals as a biological replicate.

7. Why should the test sample be freeze-dried?

In order to avoid degradation of metabolites caused by unstable metabolites of fresh tissue samples during operation, the samples need to be freeze-dried, generally using a vacuum freeze-drying method.

8. What are primary metabolites and secondary metabolites?

Primary metabolites are those necessary to maintain life activities, growth, and development, such as sugars, lipids, amino acids, nucleotides, etc. Secondary metabolites are more involved in environmental responses such as disease resistance and stress resistance. Many plant nutrition is related to functional activities that produces phenols (flavonoids, simple phenols, quinones, etc.), terpenes (carrot-like Bosu, artemisinin, saponins, picrosides, steroids), N-containing compounds (alkaloids, amines, non-protein amino acids, etc.), and others (organic acids, glucosinolates, etc.).

9. What's your requirements of samples?

Please turn to web page **Sample Requirements**.