Untargeted Metabolomics
Service Code: UTG

Summary: Survey of metabolism using relative quantitation to look for statistically significant differences in both known and unknown compounds in samples. Differences are determined based on sample factors provided by the researcher. Analytes are measured by Reversed Phase LC-MS in both positive and negative mode, and may be measured by GC-MS and Hilic LC-MS as needed.

Container: Eppendorf Tube or equivalent
Normal Volume: Plasma (200 ul) Tissue (100 mgs); Cells (2E7).
Minimal Volume: Plasma (100 uL)Tissue (50 mg); Cells (~5E6)
Special Handling: If human or primate, note any known presence of infectious agents.
Sample Collection: Snap freeze by liquid nitrogen. For tissues, resect and snap-freeze as soon as practical in tared centrifuge tube. Provide both sample weight and tared vial weight on sample submission

Reference:

Table I: IS stock solution - RP-LC and HILIC Recovery Standards

Internal Standards	Source	Cat#	IS stock solution concentration	Rt
L-^{15}N-Anthranalic acid			200 uM	
L-$^{15}N_2$- Tryptophan			200 uM	
L-D_4-Thymine			200 uM	
Gibberelic acid			200 uM	
L-Epibrassinolide			200 uM	

Table II: IS stock solution – RP-LC Injection Standards

Internal Standards	Source	Cat#	IS stock solution concentration	Rt
Zeatine			200 uM	

Table III: IS stock solution – HILIC Injection Standards

Internal Standards	Source	Cat#	IS stock solution concentration	Rt
D-13C_6_Frustose-6-Phosphate			200 uM	

Table IV: IS stock solution - GC Recovery Standards

Internal Standards	Source	Cat#	IS stock solution concentration	Rt
Myristic acid- D27			0.3 mg/mL	

Materials

1. Agilent 6520 QTOF with 1260 LC unit, chilled autosampler, with standard 54-well autosampler plate
2. Vortexer
3. Refrigerated centrifuge, capable of 15,000g with eppendorf tube compatible rotor
4. Eppendorf Vacufuge
5. ice bucket, ice
6. micro-balance
7. prepared internal standard and authentic standards mix solutions.
8. eppendorf tubes (polypropylene)
9. LCMS grade water, acetonitrile, methanol, acetone

Plasma Preparation

A. Solution Preparation

1. Create separate aliquots of plasma for LC (RP and Hilic) – 200 uL and GC-100 uL.
2. Prepare solutions for LC analysis as follows:
    a. Prepare 800 uL of crash for each sample (1:1:1 MeOH:Acetonitrile:Acetone), with 20 uL of Recovery Standards Mix (see table I) added. Final concentration of Recovery standards, 4 uM.
    b. Prepare 100 uL of 1:1 MeOH/H20 with 0.5 uL RP-LC injection standard (see table II) for each sample to be run. Final concentration of RP-LC Injection standard, 1 uM.
    c. Prepare 100 uL of 1:1 MeCN:H20 with 10 uL HILIC injection standard (see table III), final concentration of HILIC injection standard, 20 uM.
3. Prepare solutions for GC analysis as follows:
    a. Prepare 400 uL of 1:1:1 MAA containing 10 uL of GC Recovery Standard (see table IV) – final concentration of Recovery standard xxx.

B. Plasma Sample Crash/Preparation

1. For each LCMS sample of 200 µL, 800 µL MAA, 4 uM Recovery standards, is added (prepared in part **A2a**, above).
2. Vortex them at rate of 2000 rmp for 5 min in the shaker.
3. Leave the samples at 4°C for 30 min.
4. Vortex and leave them at -20°C for 1 hr.
5. Centrifuge samples vials at 15,000 rpm at 4°C for 10 min.

C. RP LC-MS Samples:

1. Transfer 200 µL of Crash Supernatant of each sample to a vial and dry the extracts at room temperature under N2 flow.
2. Add 100 µL MeOH: Water (50:50) containing RP Injection standard (made in part **A2b**, above).
3. Vortex to mix for 5 min and centrifuge them for 5 min at 15,000 rpm.
4. Transfer the supernatant to a glass 200 µL glass insert for each sample.
5. Analyze by RP LC-MS.

D. HILIC LC-MS Preparations:

1. Transfer 200 µL of Crash Supernatant of each sample to a vial and dry the extracts at room temperature under N2 flow.
2. Add 100 µL Acetonitrile: H2O (50:50) containing HILIC injection standard (F6P, 20 µM _to each sample (made in step **A2c** above).
3. Vortex to mix for 5min and centrifuge them for 5 min at 15,000 rpm.
4. Transfer the supernatant to a glass 200 µL glass insert for each sample.
5. Analyze by HILIC LC-MS.

E. GC-MS sample preparation and Derivitization

1. A 100 µL plasma sample is mixed with 400 µL MAA containing 10 µL GCMS IS myristic acid d27 (0.3 mg/ml for GC-MS in 80% MeOH), created in section **A3**, above.
2. Transfer 200 µL of supernatant to **a ependorf tube**.
3. Dry extracts in each vial at room temperature under N2 flow.
4. Prepare 20 mg/mL a solution of methoxyamine hydrochloride (MEOX) in pyridine. (e.g.: weigh 10 mg of methoxyamine hydrochloride in a glass vial and dissolve them in 500 µl of anhydrous pyridine).
5. After drying the extracts in the sample tubes, add 25 µL of the 20 mg/mL solution of methoxyamine hydrochloride in pyridine into each vial. Also add this to the blank sample and a QC sample. Cap the vials and heat them in 60 °C for 60 min.
6. After cooling the samples, 75 µL of N-methyl-N-trimethylsilyltrifluoroacetamide with 1% trimethylchlorosilane (MSTFA + 1% TMCS, Thermo Scientific) is added to each one. Put them at 60 °C for 60 min to finish the derivatizaiotn process.
7. Centrifuge samples for 5 min at 15,000 rmp.
8. Transfer supernatant to a glass 200 µL glass insert.
9. Analyze by RTL GC-MS method.