Sample preparation

Extraction solvent: MAA(1:1:1) - Methanol : Acetonitrile : Acetone with internal standards (1:25 internal standard mixture in extraction solvent).

Reconstitution solvent: Methanol:H2O (2:98)

Internal standards composition:

Name	Mass	Final Concentration, µM
L-Epibrassinolide	480.3451	184
L-[15N]Anthranilic Acid	138.0447	219
Zeatine	219.112	215
Jasmonic Acid	210.1256	217
Gibberelic Acid	346.1416	238
L-[15N]2Tryptophan	206.084	226
L-[15C]Creatinine	114.0623	217
L-(D4)Thymine	130.068	219
L-[15N]Arginine	176.1058	219
L-[D9]Palmitic Acid	265.2967	224

Procedure:

- Pipette 50uL of serum from the sample vials into a labeled 1.5 ml micro-centrifuge tube
- Add 200 µL of extraction solvent to all tubes.
- Vortex for 5 min, then let sit 30 min at 4°C. Vortex again and let sit at -20°C for 1 h, then centrifuge 10 min at 15,000 rpm.
- Transfer 200 µL of supernatant to labeled labelled 1.5 ml micro-centrifuge tube.
- Dry all samples under nitrogen stream at room temperature.
- Reconstitute in 80 µL of reconstitution solvent.
- Vortex for 5 min, centrifuge 5 min at 15,000 rpm.
- Transfer supernatant to autosampler vials with inserts, discard pellets.