

Sample preparation (Optional)

Tissue sample preparation and extraction

- A. Frozen tissue (at -80°C) was lyophilized and homogenized. About 10mg of tissue was extracted by biphasic extraction protocol, with non-polar metabolites partitioning into chloroform and polar metabolites partitioning into 70% methanol.

(Ref.: *J. Exp. Bot.* **2007**, 58, 4319-4332.

<http://jxb.oxfordjournals.org/cgi/reprint/58/15-16/4319>)

- B. Prewieghed frozen tissue was homogenized in 8 ml/g cold methanol and 2.5 ml/g cold water in homogenization tubes containing ceramic beads. The homogenates were transferred to 1.8-ml glass vials, and then additional solvents were added (8 ml/g chloroform and 4ml/g water). Samples were vortexed for 60 s, left on ice for 10 min to partition, and centrifuged as before, and then the polar and nonpolar layers were removed and dried.

(Ref.: *Anal. Biochem.* **2008**, 372, 204–212)

Blood/Plasma sample preparation

- A. Frozen plasma aliquots (stored at -80 °C) were thawed on ice. A volume of 400 μ L of organic solvent (1:1 MeOH:C₂H₅OH) containing the internal standards was added to a 100 μ L plasma aliquot, and vigorously extracted for 15 min at room temperature. The samples were then centrifuged at 124g for 10 min at 4 °C. All the supernatant was removed. The supernatant was evaporated to dryness.

(Ref.: *Anal. Chem.* **2009**, 81, 3285–3296.

<http://pubs.acs.org/doi/pdf/10.1021/ac8024569>)

- B. Frozen plasma aliquots (stored at -80 °C) were thawed on ice. A volume of 200 μ L of MeOH containing the internal standards was added to a 100 μ L plasma aliquot. After brief vortexing, serum samples were incubated for 20min at -20 °C. Supernatants were collected after centrifugation at 13200g for 10min and dried.

(Ref.: *Anal. Chem.* **2006**, 78, 743-752.

<http://pubs.acs.org/doi/pdf/10.1021/ac051312t>)

Cell sample preparation

After the cell culture, centrifuge, wash the cell pellets (at least 100mg) with ice-cold phosphate-buffered saline (PBS, pH 7.4) to eliminate the medium like sugar, etc. Then centrifuge again to get away the buffer. Add 1ml cold methanol to pellets to quench the metabolism inside the cell. Lysis the cell with ultrasonic tip or others if applicable followed by centrifugation. Use biphasic solvents (70% MeOH and chloroform) to extract and dry the supernatant. (Ref.: (*Microbiology.* **2009**, 155, 3913-3921.

<http://mic.sgmjournals.org/cgi/content/abstract/mic.0.029033-0v1> ; *Metabolomics.* **2009**, 5,199–208.

<http://www.springerlink.com/content/r3m373605q1q6581/fulltext.pdf>)