Extraction from adherent cell lines

(i) Change the medium of the cell plate(s) 2 h before metabolite extraction.

(ii) Aspirate the medium completely.

(iii) Put the plates on dry ice and add 2 ml of 80% (vol/vol) methanol (cooled to − 80 °C).

(iv) Incubate the plates at − 80 °C for 20 min.

(v) Scrape the plates on dry ice with cell scraper**.**

(vi) Transfer the cell lysate/methanol mixture to a 2-ml conical tube on dry ice **and Sonicate 10 min**.

(vii)**After incubate 1h at-20C°,** centrifuge the tube at 14,000*g* for 5 min at 4–8 °C to pellet the cell debris.

(viii) Transfer the metabolite-containing supernatant to a new 2-ml conical tube on dry ice.

(ix) Add 500 µl 80% (vol/vol) methanol (− 80 °C) to the pellet in a 2-ml tube and vortex for 1 min .

(x) Spin the tubes at 14,000*g* for 5 min at 4–8 °C.

(xi) Transfer the supernatant to a 2-ml conical tube on dry ice.

(xiii) SpeedVac/lyophilize to a pellet using no heat.

Dried metabolite samples can be stored at − 80 °C for several weeks.