Media Extraction with acetone ppt step
(07/04/17)

Acetone ppt and aliquoting

1. Weigh 50 μl media.
2. Add 200 μl ice cold ACETONE (final conc. is 80% acetone), vortex, then put samples into -80° C freezer for at least 30 min.
3. Centrifuge samples for 10 min. @ 15,000 rpm at 4° C.
4. Transfer supernatant into a pre-tared 1.5 ml snap-cap tube, record the extract mass.
5. Split the supernatant
    a. 1x ICMS (1/10 by mass)
    b. OPTIONAL 1x IC-MS (1/10)
    c. 1x NMR (remaining)
6. Lyophilize samples using a LN_2 trap.

For NMR analysis

1. Dilute the DSS (50.35 nmoles)-PO_4 (0.2 M) buffer, pH 7.4 in 100% D_2O to 1x with 1 equivalent of nanopure H_2O.
2. Dissolve lyophilized NMR fraction in 50 μl [1x DSS-PO_4 50% D_2O] buffer for loading into 1.7 mm NMR tube.
3. Load the sample into the NMR tube and measure to a minimum 35 mm height, use [1x DSS-PO_4 50% D_2O] buffer if need to bring to minimum height.

For GCMS analysis

No special instructions, process as usual.

Note: Double the amounts listed if attempting to aliquot 2 NMR fractions.
i.e. 100 μl starting media, 400 μl acetone etc.