

## Lipid Extraction with Chloroform (Serum, Plasma)

1. Take 80  $\mu$ l of the sample aliquot into 4 ml glass tube.
2. Add internal standards purchased from Avanti (17:1 LPA, 17:1 LPC, 17:0-14:1 PA, 17:0-14:1 PC, 17:0-14:1 PE, 17:0-14:1 PG, 17:0-14:1 PI, 17:0-14:1 PS, 19:0 Cholesterol ester, Sphingosine (d17:1), C17 Ceramide (d18:1/17:0), Lyso SM (d17:1), 17:0 SM (d18:1/17:0), Cholesterol (D7), Cardiolipin Mix I, Deuterated TG Mixture I and Deuterated DG Mixture I) and Steric acid - -13C purchased from Sigma.
3. Add 600  $\mu$ l of methanol, vortex.
4. Add 1000  $\mu$ l of chloroform, vortex.
5. Add 500  $\mu$ l of water, vortex.
6. Centrifuge at 3000 R for 10 min.
7. Collect the lower (chloroform) phase.
8. Add additional 600  $\mu$ l of chloroform, repeat step 6 to step 7.
9. Evaporate the combined organic phases to dryness in a vacuum centrifuge.
10. Reconstitute extracted lipids in 100  $\mu$ l of IPA/methanol (50:50) for storage.