

Preparation of plasma/serum and tissue samples for oxidized lipid LC-MS/MS analysis.

All samples must be prepared with added “anti-oxidant” Butylated Hydroxytoluene (BHT) and overlayed with Argon gas (or Nitrogen gas if Argon gas is not available) prior to storage at -80°C. Sample should be shipped on dry ice.

BHT (molecular weight 220.35) is purchased from Sigma Aldrich - catalog# B1378-500G

For a final concentration of 100µM BHT in plasma/serum samples:

Make a 1000x stock of BHT in EtOH (100mM) = 220.35mg BHT dissolved in 10ml ethanol.

Store 500µl aliquots of this 1000x stock in low retention siliconized eppendorf tubes at -20°C.

For addition of BHT to plasma/serum, from the 1000x BHT stock make the required volume of a 100x BHT stock in PBS (10mM) = 5µl 1000x stock in 45µl PBS. Vortex this 100x BHT stock briefly to mix and then add the required amount to plasma/serum (1µl 100x BHT stock per 100µl plasma/serum). Vortex plasma/serum sample briefly after addition of BHT, spin down briefly, overlay with Argon gas, apply “parafilm” around tube/closure and store samples at -80°C.

For tissue homogenates:

Homogenize tissue piece (approximately 200mg weight is ideal) in 2ml PBS containing 50µM BHT (prepared by adding 250µl of 1000x BHT ethanol stock to 500ml PBS). Store 500µl homogenate aliquots under Argon at -80°C (as for plasma/serum samples above)).

For HODE/HETE LC-MS/MS analysis, 500µl of tissue homogenate (prepared from ~200mg tissue as described above) or minimum 50µl (ideally 100µl) plasma/serum is required.