Murine Liver Extraction Protocol for Acyl-Carnitines

1. Weigh out 35-40mg of frozen homogenized murine liver tissue into screw-capped vials and refreeze the aliquots in liquid nitrogen.
2. Perform extractions in triplicate; thus 3 aliquots of each liver sample are required.
3. Add 1.5mls of solvent to liver tissue aliquots; isopropanol (IPA) or hexane:IPA (50:50v/v) works best for overall acyl-carnitine extraction.
4. Add (15-20) 1.0mm silica beads to each tube. Prepare a method control using an equal volume of solvent and beads only.
5. Homogenize all samples: 3x30 seconds @ 6500 rpm in Precellys 24 tissue homogenizer.
6. Centrifuged mixture at 14,000*xg* for 10min at 4^{o}C to precipitate particulate matter.
7. Transfer supernatant to clean tubes and dry in speed-vac.
8. Solubilize residual material via sonication for 15 minutes in 400ul 50:50 MeCN/ H_2O + appropriate internal standard. (^{3}H-palmitoylcarnitine, $^{13}C^{15}$N-AMP, ect)
9. Centrifuge 14k rpm for 10 minutes @ 4^{o}C to pellet insoluble debris.
10. Transfer supernatant to auto sampler vials.