**GC-MS protocol for fatty acid methyl ester**

1. 20-50 µl serum (50 µl) or 20-50mg liver tissue (~30mg)
2. Add 10-15 beads and 1ml MeOH/CHCl3 (2:1) with 50 uM internal standards **(C15-acid and C17-methyl ester as internal standard, 5ul of 10mM)** (Prepare one blank and internal standard)
3. Homogenized 6500 rpm for 2\*20 seconds (2-3times), then vortex 3 minutes, Centrifuge 15 mins at max speed.
4. Transfer supernatant into 2 ml EP tubes; **add 500ul saline (0.9%)**
5. Votex 5 mins, Centrifuge 15 mins at max speed;
6. Top layer (aqueous) and bottom layer (organic)

**Top**

1. Transfer to 1.5 ml tube, dry down in speed vacuum for 3-4 hours
2. Add 100 µl MOX, incubate overnight at room temperature
3. Take 50 µl supernatant, Add 50 µl BSTFA (10% TMCS), incubate 1 hour at 60°
4. transfer to vial for GC-MS

**Bottom**

1. Transfer to 10 mL glass tube, dry down in N2;
2. **Add 1 mL MeOH/HCl (41.5ml/9.7ml), vortex 5mins, incubate overnight at 60°;**
3. Add 5mL hexane and 5 mL saline (0.9%), vortex 1 mins;
4. Take supernatant of hexane fraction, dry down in N2
5. Add 200 µl hexane, vortex 1 mins
6. Transfer to vial for GC-MS
7. **Note:**
8. Fluorophenyl actylglycine MW=211.19 g/mol, 10mM stock in 10mL (21.1mg)
9. Tridecanoic acid MW=214.34 …… (21.4mg)
10. Heptadecanoic acid MW=270.45 …… (27.0mg)
11. Nonadecanoic acid MW=298.50…… (29.8mg)
12. **All above solution 5 µl in 1mL = 50 µM**