**Tissues extraction for NMR metabolomics**

1. Weigh ~50 mg tissues and make a record; mark and save on ice;
2. Add 8-10 beads and 1 ml solution (pre-cooled methanol:H2O (2:1) ) to homogenization tubes, Vortex 10s;
3. Homogenization with 6500 – 1x20 – 005 program for 2-3 times;
4. Vortex 1mins and incubate 5mins at room temperature;
5. Centrifuge at 4 0C, 11180g for 10mins;
6. Transfer the supernatants to 2 ml EP tubes;
7. Add 0.6 ml solution (methanol: H2O (2:1)) to the pellets**, repeat the above procedure (4-6 steps)**.
8. Combine the supernatants;
9. Dry down and then save it in -800C refrigerator.

10)0.6 ml 0.10M PBS (D2O) dissolve it, centrifuge at 4 0C for 10mins, then transfer 0.55ml supernatants to NMR tubes.

**Plasma or serum for NMR**

1. 200 µl samples mixed with 400 µl PBS (0.045M) containing 50% D2O in 0.9% saline;
2. Centrifuge at 18100 g for 10 minutes;
3. Transfer 550 µl supernatants into NMR tubes.

**Cecum content and Feces extraction for NMR metabolomics**

1. Weigh 50~60 mg samples and make a record; mark and save it on ice;
2. Add 8-10 beads and 1.0 ml **PBS** (0.10M) solution containing 50% D2O to homogenization tubes, Vortex 30s;
3. Homogenization with 6500 – 1x20 – 005 program for 2-3 times;
4. **Freeze-thawing two times with Liquid nitrogen;**
5. Centrifuge at 4 0C, 11180g for 10mins;
6. Transfer the supernatants to 2 ml new EP tubes;
7. Add 0.6 ml **PBS** solution to the pelletsfollowed with Vortex 30s and Centrifuge at 4 0C, 11180g for 10mins. **(no dry down step)**
8. Combine the supernatants, centrifuge at 4 0C and 18100 g for 5 mins; Transfer the supernatants (0.55 ml) to NMR tubes.

**Preparation of PBS** **solution containing 50% D2O**

For example: **0.1M** Na+/K+ PO4 Buffer (**100ml**, pH=7.4)

K2HPO4**.**3H2O: 0.8\*100(ml)\*0.1(M)\*228.22\*10^(-3)/0.99=1.8442 g

NaH2PO4**.**2H2O: 0.2\*100(ml)\*0.1(M)\*156.01\*10^(-3)/0.99=0.3152 g

H2O: 100ml\*0.5=50 ml

D2O: 100ml\*0.5=50 ml

TSP: 0.002% (w/v)\*50=0.001 g