**Tissues extraction for NMR metabolomics**

**Materials:**

* NMR Tubes (5mm TC-5-EVA-P)
* PBS (K2HPO4:NaH2PO4=4:1)
* D2O (deuterium oxide)
* TSP (trimethylsilyl propanoic acid)
* Homogenization Tubes
* Acrylic Beads
* Methanol
* Water

1. Weigh ~50 mg tissues and make a record; mark and save on ice;
2. Add 8-10 beads and 1 ml of pre-cooled methanol:H2O (2:1) to homogenization tubes, Vortex 3s;
3. Homogenization with 6500 – 1x20 – 005 program for 2-3 times;
4. Incubate 5 min at room temperature;
5. Centrifuge at 4ºC, 11180 g for 10 min;
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 at -80ºC.
10. Resuspend in 0.6 ml **0.1 M PBS** (D2O), centrifuge at 4ºC for 10 min, then transfer 0.55 ml supernatants to NMR tubes.

**Preparation of 0.1M PBS** **solution containing 50% D2O**

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

K2HPO4: 0.8\*100 (ml)\*0.1(M)\*174.18/1000/0.99 = 1.408 g

NaH2PO4: 0.2\*100 (ml)\*0.1(M)\*119.98/1000/0.99 = 0.242 g

H2O: 100 ml\*0.5 = 50 ml

D2O: 100 ml\*0.5 = 50 ml

TSP: 0.005% (w/v)\*100 = 0.005 g

NaN3: 0.01 g (Preservative)

**Plasma or serum for NMR**

If the volume of serum or plasma is enough:

1. 200 µl samples mixed with 400 µl **PBS** (**0.045M**) containing 50% D2O in 0.9% saline;
2. Vortex samples for 10 s;
3. Centrifuge at 18100 g for 10 minutes, 4ºC;
4. Transfer 550 µl supernatants into NMR tubes.

If the volume of serum or plasma is not enough (≤ 50 µl):

1. 30 µl samples mixed 30 µl **PBS** (**0.045M**) containing 50% D2O in 0.9% saline;
2. Vortex samples for 10 s;
3. Centrifuge at 18100 g for 10 minutes, 4ºC;
4. Transfer 60 µl supernatants into **1.7 mm** NMR tubes with microsyringe.
5. Seal and label the 1.7 mm NMR tubes, put them into 5 mm NMR tubes. (NS = 256 when acquire 1H NMR experiments)

**Preparation of 0.045M PBS** **solution containing 50% D2O in 0.9% saline**

**0.045M** Na+/K+ PO4 Buffer (**100 ml**, pH=7.4)

K2HPO4: 0.8\*100(ml)\*0.045(M)\*174.18/1000/0.99 = 0.633 g

NaH2PO4: 0.2\*100(ml)\*0.045(M)\*119.98/1000/0.99 = 0.110 g

0.9% saline: 0.9 gm in 50 ml water (+50 ml D2O)

D2O: 100ml\*0.5 = 50 ml

TSP: 0.005% (w/v)\*100 = 0.005 g (no TSP, which will react with protein in serum or plasma!!!)

**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 (0.1M)** 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 4ºC, 11180 g for 10 mins;
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 4ºC, 11180g for 10 mins. **(no dry down step)**
8. Combine the supernatants, centrifuge at 4ºC and 16099g for 10 mins; Transfer the supernatants (0.55 ml) to NMR tubes.

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

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

K2HPO4: 0.8\*100 (ml)\*0.1(M)\*174.18/1000/0.99 = 1.408 g

NaH2PO4: 0.2\*100 (ml)\*0.1(M)\*119.98/1000/0.99 = 0.242 g

H2O: 100 ml\*0.5 = 50 ml

D2O: 100 ml\*0.5 = 50 ml

TSP: 0.005% (w/v)\*100 = 0.005 g

NaN3: 0.195 g (Preservative)

**Urine for NMR**

1. 200 ul samples mixed with 400 ul PBS (1.5M) containing 50% D2O;
2. Vortex samples for 10s;
3. Centrifuge at 18100g for 10 minutes, 4ºC;
4. Transfer 550 ul supernatants into NMR tubes

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

**1.5 M** Na+/K+ PO4 Buffer (**100 ml**, pH=7.4)

K2HPO4: 0.8\*100 (ml)\*1.5(M)\*174.18/1000/0.99 = 21.113 g

NaH2PO4: 0.2\*100 (ml)\*1.5 (M)\*119.98/1000/0.99 = 3.636 g

H2O: 100 ml\*0.5 = 50 ml

D2O: 100 ml\*0.5 = 50 ml

TSP: 0.005% (w/v)\*100 = 0.005 g