组织样品

Normalized by weight

Make sure <10% error of weight across different samples before extraction

1. Cut tissue on dry ice and record the tissue weight, make sure <10% error across different samples; (干冰上切割称重或液氮中研磨后称重)
2. 20 mg tissue + 200 uL H_2O to homogenized， homogenize for three cycles (each cycle: 5500rpm for 20s,repeat three times) with cooled N_2 gas flow from liquid N_2
3. 200 uL 匀浆液+ 800 uL MeOH/ACN (v:v, 1:1)
4. Vortex 30 s, sonicate 10 min (4 ℃ water bath)
5. Incubate 1 h at -20 ℃ (fascinate protein precipitation)
6. Centrifuge 15 min at 13000 rpm and 4 ℃
7. Take supernatant and evaporate to dryness at 4 ℃ using a vacuum concentrator
8. Reconstitution: 100 uL of ACN /H2O (v:v, 1:1)
9. Vortex 30 s, sonicate 10 min (4 ℃ water bath)
10. Centrifuge 15 min at 13000 rpm and 4 ℃， placed in -80 ℃