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# Metabolomics Extraction from Stool Samples

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https://www.57357.org/proteomics-unit/

1. Store collected fecal samples at -80°C until usage.
2. Thaw samples on ice.
3. Take 100 mg from each sample in new Eppendorf.
4. First Extraction:
    - Add 400 µl of extraction solvent (acetonitrile: methanol, 3:1) to the samples.
    - Vortex for 2 minutes.
    - Sonicate for 5 minutes.
    - Centrifuge for 10 minutes at 4°C at 10,000 rpm.
    - Transfer the supernatant to new Eppendorf.

5. Second Extraction: (Repeat the above steps)
    - Add 400 µl of the extraction solvent to the pellet and homogenize.
    - Sonicate for 15 minutes.
    - Centrifuge for 10 minutes at 4°C at 10,000 rpm.
    - Transfer the supernatant to the Eppendorf of the first extraction step.

6. Speed-vac the supernatant at 30°C.
7. Reconstitute in reconstitution buffer (water: methanol: acetonitrile, 2:1:1) at ratio 1mg/1ml.