# Sample preparation for plant tissue

Freeze plant tissues with liquid $N_2$

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Grind plant tissue into powder with a mortar and pestle in liquid $N_2$.

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Weighing 300~500 mg (fresh weight) plant tissue and keep the samples in liquid $N_2$.

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Add 1.125 mL of a mixture cold $CHCl_3$/MeOH (2/1)

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Mix sample for 15 min at 4°C

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Add 375 µl cold H2O

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Mix sample for 15 min at 4°C

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Centrifuge at 13,000 rpm for 10 min at 4°C

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Move the upper (hydrophilic) layer to new eppendorf

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Move the lower (hydrophobic) layer to another eppendorf (Pierce the protein disc carefully)

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Freeze by liquid $N_2$

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Dry out by SpeedVac and keep at -80 ℃

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(before LC-MS analysis)

Reconstitute the hydrophilic layer with 180~300 µl MeOH:H2O (1/1) and hydrophobic layer with CHCl3:MeOH (2/1)

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Centrifuge at 13,000 rpm for 15 min at 4°C and take the supernatant