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### **Nitric acid digestion protocol**

- (1) Dry the sample at 70°C for 2 days.
- (2) Digest about 10 mg of dried sample in a mixture of 600 µL hydrogen peroxide (30%) and 1200 µL nitric acid (60%) at 140°C for 4 h (Cheng L. et al., 2007).
- (3) Dilute the digested sample with water, and filtered using a 0.22-µm filter.
- (4) Measure the ion contents using atomic absorption spectrometry (AAS), inductively coupled plasma - atomic emission spectrometry (ICP-AES), or inductively coupled plasma - mass spectrometry (ICP-MS).

### **Reference**

**Cheng L, Wang F, Shou H, Huang F, Zheng L, He F, Li J, Zhao FJ, Ueno D, Ma JF, et al**  
(2007) Mutation in nicotianamine aminotransferase stimulated the Fe(II) acquisition system and led to iron accumulation in rice. *Plant Physiol* **145**: 1647–1657