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# Microwave digestion for microbes

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1 Works for me

 Share[dx.doi.org/10.17504/protocols.io.4r3l2747xg1y/v1](https://dx.doi.org/10.17504/protocols.io.4r3l2747xg1y/v1) An.Huang

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## ABSTRACT

Before conducting inductively coupled plasma mass spectrometry (ICP-MS), all samples containing organic compounds need to be digested in order to eliminate all organic parts. Microwave digestion is a commonly used method for organism digestion. This protocol describes how to conduct microwave digestion on microbes.

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## PROTOCOL CITATION

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MATERIALS TEXT

Samples to be digested, digestion vessels (with caps and plugs), nitric acid (70%), hydrogen peroxide (30%), microwave digestion system, ddH<sub>2</sub>O.

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## Preparation of digestion vessels

1h

- 1 Pick out digestion vessels from preservation boxes containing nitric acid (4%). Rinse and wash the vessels thoroughly. Also wash coordinated number of caps and plugs.

Digestion vessels should be kept in acid solutions. The vessels should be washed thoroughly, especially the inside bottom of the vessels. Because any elements left inside the vessels will dramatically influence the following analysis result.

Washing the vessels using ddH<sub>2</sub>O and sterilized distilled water (SDW) in turn for three times could barely leave any interfering elements remaining at the bottom.



Be careful with preservation boxes since they contain oxidative acid.

2 Put all vessels inside the oven and dried at **60 °C** for at least **01:00:00** .

1h

3 Take all vessels out from the oven when they are completely dried and put all vessels into the fume hood.

#### Microwave digestion

4 Label all vessels according to the samples.

5 Add samples into digestion vessels.

We use a 200 µL pipette to add our samples dispensed in liquid. Calculate the volume or mass to be added in the vessel according to the following analysis method.

6 Add **5 mL** nitric acid (70%) and **2 mL** hydrogen peroxide into the digestion vessels.

Both reactants need to be more than adequate to ensure thorough digestion of organic compounds.



Be careful with both reactants. Nitric acid (70%) is corrosive and can cause serious burn. Hydrogen peroxide (30%) may explode when heated or mixed with nitric acid.

7 Plug the vessels and screw the caps tightly.

7.1 Here we skipped a step of pre-digestion, which is to heat these capped vessels at 80°C overnight. Since our samples only contain microbes like bacteria, it is

not a necessity to conduct this step. This part is designed for larger organisms like cellulose, which is hard to digest.

- 8 Put all vessels into microwave digester according to the manual of the digestion system.
- 9 Set the digest programme according to the vessel number. Please read the digestion system manual for more instruction.
- 10 When the digestion programme has finished, wait for around half an hour to let vessels cool down.


#### Volume consistency

- 11 Open the caps and unplug the vessels when the vessels have not completely cooled to room temperature. Pour the remaining liquid inside the vessel into a 50 mL centrifuge tube.

The vessels may be extremely difficult to open if left to cool down to room temperature. Make sure conducting this step before they completely cool down.




The vessels are pressure vessels. Do not open the cap upon programme finishing. Wait for a few minutes.

- 12 Use a dropper to add  3 mL ddH<sub>2</sub>O into one vessel by the inside wall. Shake the vessel generally and pour the water into the same 50mL centrifuge tube in Step#11. Repeat this step for 6 to 8 times.

This step makes sure that all digested matters remaining inside the vessels are washed off into the 50 mL tube. This reduces the loss of elements during digestion.

- 13 Add ddH<sub>2</sub>O until the liquid volume inside the 50 mL centrifuge tube reads  **50 mL** .

This makes all samples digested share a same volume, which simplify the following analysis regarding elements amount.

- 14 The digested samples in 50 mL tubes need to be stored at  **4 °C** for further analysis.

#### Digestion vessel storage

- 15 Wash all digestion vessels using ddH<sub>2</sub>O for three times.
- 16 Submerge all vessels into nitric acid (4%) inside the preservation box.