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ICP-MS: preparation and nitric acid-assisted digestion of plant material

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We use this protocol and it's working



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Abstract

This protocol is a guided, step-by-step instruction for the digestion of diverse plant materials in preparation for element analysis with inductively coupled plasma mass spectrometry (ICP-MS) using concentrated nitric acid (HNO₃). Useful tips and comments are given and are placed throughout the text via yellow note boxes. This protocol covers the sampling, reagent addition, and heating parameters in preparation for the initial digest. Following digestion, samples are diluted and centrifuged to remove particles, resulting in a clear, particulate- and precipitation-free solution suitable for ICP-MS analysis.

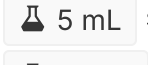
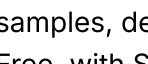
Guidelines

- preparation of empty tubes
- sampling: harvest, weighing, and filling the tubes
- digestion of the samples with HNO₃ (takes about 3 days)

GOAL: the release of elements of interest in a final clear, particle-free solution suitable for measurement with an ICP-MS.

REMINDER: The calculated dilution factor is needed at the end of this protocol to be applied on the measured ICP-MS data.

Materials

- Concentrated (67 - 69 %) HNO_3 (nitric acid), suitable for trace analysis in ppb range (e.g. Sigma Aldrich Nitric acid 69%, Suprapur - 1151871000)
-  1.5 mL sample tubes (good for small amounts of material [around 10 mg] or material that need to be grinded)
-  2 mL sample tubes (is sufficient in most cases, especially for the digest of [10 - 20 mg] powdered samples)
-  5 mL sample tubes (useful for lager amounts of material [over 20 mg] that need to be digested)
-  15 mL conical centrifuge tubes, metal-free (tubes for the mixing with water and another set for the final samples, depending on the ICP-MS sample system, this can also vary) (e.g. VWR, Centrifuge Tubes, Metal-Free, with Screw Cap - 525 0629)
- MilliQ water/ de-ionized water/ ultra pure water
- Heating block or water bath
- Sample tube holder and conical centrifuge tube holder
- **OPTIONAL:** Multi-stepper pipette (makes the life easier in the dilution step)
- Centrifuge (up to 12.000 g) - If possible, adjustable to 4°C, as we have found that this reduces the precipitation of samples further on.

Safety warnings

-  **Danger**
 strong acid - please take note of the safety data sheets
 burns due to high temperatures

Before start

IMPORTANT: Please remember and remind yourself about potential safety measures before starting this protocol. Depending on the sample type and the amount of material used, variations can occur in this protocol. The variations are indicated through the comments and notes and examples for these variations are given. This protocol is created for the preparation of plant material but other materials can also be measured using ICP-MS, such as soil and animal material (other protocols are needed for these materials).

IN GENERAL, THE FOLLOWING APPLIES TO THIS PROTOCOL: The goal is to create a clear, particle-free sample solution suitable for ICP-MS without introducing foreign elements of interest. While this protocol provides general guidance for the procedure, modifications and adjustments may be required depending on the plant material, available equipment, and experimental conditions.



Preparation of tubes

- 1 Three sets of tubes are needed for this protocol. The digest is done in two sets and the final samples are stored in the last set.

Note

To see which tubes are suitable for different amounts of plant material, see the list under "Materials" with examples for the different tube types.

- 2 Label all sets and weigh one tube set in which the digest will be done. Note down the **empty weight**.

Note

In the end, you will have three sets of tubes. The **first** set is used to weigh in the samples and add the first unit of acid. The **second** set is weighed before sample addition and again after digestion; digestion is also continued in this set. The **third** set is used to store the final sample solution.

We, for example, usually start with sample tubes with either a  1.5 mL or  2 mL volume as the first tube set, and then switch for the second and third set to  15 mL conical centrifuge tubes during digestion.

- 2.1 Prepare a blank tube.

Note

CRITICAL: A blank is handled exactly the same as your samples except that there is no plant material present, to see if any contaminations are introduced during the protocol.

- 3 Create an Excel file with your samples names, and fill in the empty weight, and later the filled tube weight.

Note

You can also use another data organization tool that is at your disposal. In our case, we used Excel, but this is not mandatory.

Sampling

- 4 Harvest the targeted plant material and ensure that no contaminants of your elements of interest are introduced (e.g. soil, chemicals, agar, other plant material or contamination from laboratory equipment).

Note

The exact procedure of the sampling varies according to the plant material. Please think about the type of sample material you are planning to use beforehand. At the end of the protocol, the dry weight of the plant material and the weight of the final sample solution are required to calculate the dilution factor.

- 4.1 If material has to be pulverised, place the sample material in tubes and use, for example, a Retch machine for grinding with metal-free ceramic balls; metal-free mortar and pestle can also be used.

Note

- Extreme fine or small sample material such as *Arabidopsis thaliana* seeds or small leaves should not be ground, since sample material is lost in this process.
- Please don't use glass balls since these tend to break and if you cannot use metal balls because this is an element of interest for you, use ceramic balls instead.
- The grinding step serves to increase the material surface area of the material for subsequent acid digestion.
- In general, the following applies: increase the surface area of the plant material using by all means available without introducing contamination from elements of interest.

- 5 Dehydrate the samples until complete dryness so that no weight fluctuations are observed. Drying in the oven at around  65 °C can take a couple of days.

Note

- The drying of sample plant material can be carried out in tubes, plates, or any other way that prevents the elements of interest from coming into contact with the plant material.
- *Arabidopsis thaliana* leaves and seeds take in general  72:00:00 at  50 °C in a sample tube with an open lid.
- Complete dehydration is necessary to achieve comparability between different plant materials, as residual moisture may cause variation depending on the material and the ambient humidity at the experimental site.



- 6 Transfer approximately  10 mg -  20 mg of (ground) plant material into pre-weighted sample tubes (first tube set) and note down the weight in the Excel file.

Note

With this recommended material amount we had the most success, but samples with higher amount can also be digested. You only have to plan for this accordingly, for example with bigger sample tubes and later on more acid.

- 7 Calculate sample dry weight: Sample DW [mg] = final tube weight with sample material - empty tube weight.

Note

The samples can also be weighed before adding them to the tubes; at the end, only the accurate dry weight needs to be available in order to calculate the dilution factor.

Digestion with HNO₃

2d 3h 15m

- 8 Add to each sample  0.5 mL concentrated nitric acid (HNO₃) and incubate (pre-digest)  Overnight at  Room temperature .

1d



Note

- If more than  20 mg material is used,  1 mL nitric acid and a bigger tube, such as a  5 mL tube, is necessary to prevent overflow while heating up the acid.
- Digest of whole plant material may increase the incubation time, e.g. *Arabidopsis thaliana* seeds or young leaves can be digested without grinding and are incubated for at least 1 or 2 days.

- 9 Place samples on  95 °C in a heating block or water bath for at least  00:30:00 or till the liquid is completely clear. Prevent pressure generation within the tubes by using open lids in the heating block, or using loosely tighten screw caps in the water bath.

30m

**Note**

- Prevent sample dilution from unwanted water due to condensation, when using the water bath.
- Heating time of the digest may vary for different plant materials; e.g. grinded beans, such as *Vigna radiata*, can take up ⌚ 02:00:00 to ⌚ 03:00:00 . Always keep an eye on your sample and check every ⌚ 00:30:00 till the sample solution is completely clear.
- Depending on the plant material, the samples can foam and overflow when heating up in acid. Keep an eye on the samples. Especially if the plant material is known for foam formation, plan accordingly with bigger tubes, less acid etc.
- If the sample does not digest even after a long time, H₂O₂ can be added to increase digestion. However, this definitely causes the samples to foam.

10 After the digest, let the samples cool down to 🌡 Room temperature for at least ⌚ 00:15:00 .

15m

11 Prepare the second tube set, 🧴 15 mL conical centrifuge tubes (pre-weighted), with 🧴 4.5 mL Milli-Q water (using multi-stepper pipette, if available) and transfer the complete supernatant into their respective 🧴 15 mL conical centrifuge tubes via pipetting or decantation (!!! **exothermic reaction** !!!).

Note

Mixing water and HNO₃ is an **exothermic reaction**. To avoid splashing due to excessive heat generation, the acid should be added carefully to the water.

12 **CRITICAL:** !!! the final acid concentration shouldn't be above 5 % to prevent damages of the ICP-MS machine !!!

13 Weigh the samples in their conical centrifuge tubes and note down the final weight in the Excel file.

14 Calculate the dilution factor with the weights of the second tube set: $DF = ((\text{final weight of the conical centrifuge tube [g]} - \text{empty conical centrifuge tube weight [g]}) * 1000) / \text{sample DW [mg]}$.

**Note**

- The dilution factor is later applied on the ICP-MS data to get the real concentration of the elements of interest.
- The difference in units between the milligrams  0 mg of plant material and the grams  0 g of tubes must be taken into account in the calculation, using the multiplication factor 1000. If all values were measured in the same unit, this adjustment can be omitted from the calculation.

- 15 Store the conical centrifuge tubes at least  Overnight at  4 °C .
Samples can also be stored longer at  4 °C if needed.

1d



- 16 Centrifuge the samples at  12000 x g, 4°C for at least  02:00:00 .
Alternatively, filtrate the samples.

2h

**Note**

- If there are problems with precipitation of particles from the samples, the speed of the centrifuge can be increased depending on the capabilities of the conical centrifuge tubes used (see manufacturer notes).
- If it is not possible to use a centrifuge with a cooling function, the samples can also be pre-cooled for a period of time at around  4 °C and then be centrifuged at  Room temperature . But we noticed that this may lead to an additional centrifugation step later on to remove possible precipitation that occurs more often.
- The longer the centrifugation step the better.
- The sample material should be kept cold to prevent precipitation of the samples.
- When filtrating the samples, fibers of the filter may enter the solution, depending on the material used in the filters. As such we prefer the centrifugation methode.

- 17 Transfer about  4 mL of the supernatant into the respective  15 mL conical centrifuge tube of the third tube set (not weighted).

Note

- The final sample should be a clear, partical-free and precipitation-free sample solution to prevent the clogging of the ICP-MS spray chamber.
- The tubes containing the supernatant and residual nitric acid must be disposed in accordance with local regulations for the handling and disposal of acidic waste.

- 18 Store the samples at  4 °C until they can be measured by ICP-MS.

**Note**

Please keep in mind that the samples cannot be stored forever, since some of the elements of interest are not stable over a long period of time.

18.1 Final check before starting ICP-MS measurements.

30m

Note

Check your samples before measuring by ICP-MS, since the samples can precipitate even when stored at  4 °C . If this happens, centrifuge your samples again beforehand at  4 °C for at least  00:30:00 and transfer the supernatant to a new conical centrifuge tube.

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