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NECi Protocol for Nitrate Analysis in Plant Tissues: Laboratory Methods

Much of the nitrate in plant tissues is held within the cell walls. In order to determine total nitrate it is necessary to break the cells open. There are many standard techniques in use, including freezing and crushing the tissue, grinding in a blender, boiling, and combinations of these techniques. Here is a simple boiling method we've used with our kits. The standard ratio is one gram of sample per 10 milliliters of water (or extraction buffer).

Many plant tissues - especially root and leaf vegetables - can have nitrate at very high levels. Carrots and corn stalks may have 500 ppm or more. You will need to dilute these samples before analyzing them with our Nitrate Test Kits. Dilute extracts 1:10 and 1:10 again (using water or buffer), and analyze both dilutions.

For Quantitative work, dry samples should be used. You will find instructions for drying samples to constant weight on the reverse of this sheet.

1. Prepare a set of containers that can withstand boiling. Suitable containers include 50 ml centrifuge tubes or 25 ml Erlenmeyer flasks. Label one for each of your samples.
2. Cut each sample into small pieces. Place **one gram** of each sample into its labeled container. Add **10 ml** of deionized water (or extraction buffer) to each container.
3. **Optional step:** Add 50 mg activated charcoal to each flask. This step is required for quantitative data at levels below 1.0 ppm nitrate-N (below 70 µM nitrate; Low Range kits), and is helpful for samples with high sugar content.
4. Set the labeled containers in a rack in your water bath. If a water bath is not available, use a pan of water on a hot plate. Be sure the keep flasks or other containers from tipping over. Bring flask contents to boil, and boil for 20 minutes. Cool to lukewarm before proceeding.
5. Decant the liquid contents of each container into a new vessel. Collect as much residual liquid as possible.
6. Bring volume back up to the initial 10 ml volume by adding deionized water. Mix.
7. **Optional step:** Clarify by filtration, preferably using a syringe filter. This is helpful for quantitative work, and necessary when analyzing samples below 1.0 ppm.
8. Samples are ready to be analyzed. Samples can also be stored in –20°C freezer until analysis. You will need 50µl sample for Standard range kits (10µl for microplate); use 500 µl sample for Low Range kits (50 µl for microplate).

Proceed to Nitrate Analysis. See *Screening Method* for simplified instructions for School, Home or Farm nitrate measurement.

Suggested Protocols for Drying Samples to Constant Weight

Plant tissues, Soil Samples, Compost Samples, etc.

For quantitative work, it is often necessary to dry samples to constant weight. This is required, for example, when expressing nitrate content as a percentage of the weight of the sample. Constant weight is obtained by removing the water from the sample - that is, by drying it. Here are two procedures for drying samples to constant weight.

Once the samples are dry, use one gram of the dry sample and extract for nitrate using your standard protocol, or use a protocol provided by NECi.

I. Standard drying method

1. Take a sufficient amount of each sample to be sure to have more than 1 gram of dry weight. Exact weight is not important at this stage. Be **sure** to record this weight.
2. Place samples in an oven at approximately 60°C. Weigh at 24 & 48 hours. Samples are dry when the weight is constant for two consecutive readings. The time required will vary with the water content of the sample.
3. Once a constant weight value is obtained, the samples are ready for analysis.

Note: If a vacuum oven is available, the drying time can be shortened considerably.

II. Microwave drying method

1. Weigh samples to be dried.
2. Spread the weighed samples onto microwave-safe containers. Glass or many plastics are fine. If using a paper plate or paper bag, be **sure** to include water in the microwave oven as described in Step 3. Spread the samples as a thin layer to aid the drying process, and place them in the microwave oven.
3. Place an 8oz glass (or 250 ml beaker or flask) three-quarters full of water in the oven. This should prevent the samples or paper plates from igniting in the microwave oven.
4. Heat at 80 per cent of maximum power (or use a medium high setting) for 4 minutes.
5. Remove the sample, stir and weigh it.
6. Continue to reheat for 2 minute intervals, re-weighing each time. To prevent burning, use lower heat and 30 second time intervals as samples approach dryness.
7. When the weight of the sample does not change after two drying intervals, it is dry. A slightly charred sample will not affect accuracy of the nitrate assay, but if the sample burns, the drying procedure should be repeated.
8. Once a constant weight is obtained, the samples are ready for analysis.