

Plant Petiole Nitrate Test Kit Instructions

Introduction

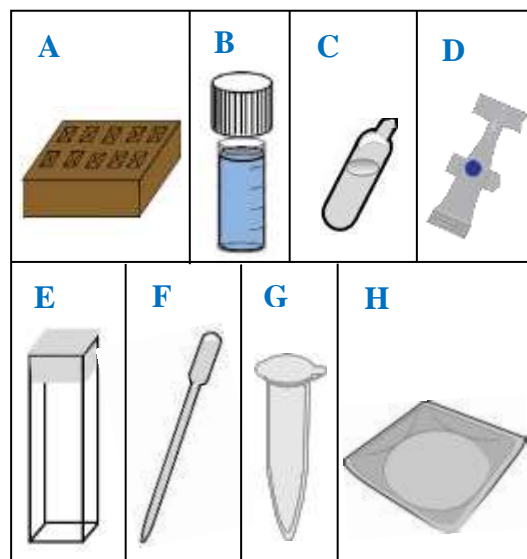
This kit contains everything needed to test plant samples for nitrate content. Provided in this kit is a **500 ppm Nitrate-N standard (D)**, to use as a reference tool for analyzing samples and to ensure that the kit is working properly. Download the free "Nitrate Color Slider" application, available on Google Play and iTunes App, for easy analysis of your samples on mobile devices. A color chart is also provided on the back of this instruction sheet for sample analysis. These kits are based on validated laboratory methods, and will provide nitrate results after 15-20 minutes. Although results are not as precise as a testing lab, *you will get accurate nitrate content results* for making preliminary decisions about nutrient application.

Kit Contents (per 5 samples):

- A.** 1 cardboard cuvette holder
- B.** 5 clear collection tubes with white screw caps containing 5 mL water
- C.** 5 small squeeze bulbs (contains buffer)
- D.** 1 large squeeze bulb with blue dot (contains **nitrate standard**)
- E.** 6 square reaction cuvettes in sealed foil pouch (contains enzyme)
- F.** 10 plastic pipettes (for transferring one drop of liquid to containers)
- G.** 6 snap-cap tubes of color reagent powder (in amber bag)
- H.** 5 plastic pans for plant sap collection

Materials you will need:

- Marker for labeling tubes and cuvette caps
- Scissors (to remove tip from *snip off* squeeze bulb)
- Pen (for recording data on data sheet)
- Garlic press, clean pliers, or similar apparatus to extract plant sap

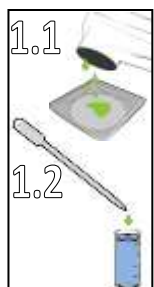


DO:

- ✓ Store this kit in a cool and dark place (below 72°F/22°C), refrigerate if possible
- ✓ Follow the directions of a Certified Crop Advisor for advice on plant sampling. A common method for plant testing calls for extracting sap from the petioles. Contact NECi for more information.
- ✓ Run tests and standard all at once to ensure accuracy
- ✓ Label collected sample tubes and reaction cuvettes accordingly
- ✓ Gently shake cuvettes several times throughout reaction without inverting them.

DO NOT:

- *Open sealed packets or mix cuvette contents until ready to use
- *Add more than *one drop* of sample water to reaction cuvette
- *Compare samples to color chart before waiting at least 10 minutes
- *Invert reaction cuvettes when mixing or get liquid in the cap portion before step 4

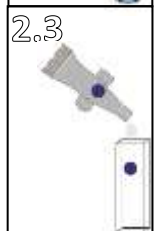


Procedure

Step 1 Sample Preparation

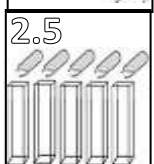
- 1) Use a garlic press or clean pair of pliers to squeeze several drops of plant juice into plastic pan. (1.1)
- 2) Repeat for each plant sample, using a clean pan for each. *Clean press or pliers between samples.*
- 3) Add one drop of plant juice to each tube with water using a new pipette for each sample. (1.2) Label Tubes
- 4) Recap the tubes and shake each tube for approximately 1 minute.

**Be sure to label each cap and record the information regarding the samples on the data sheet*



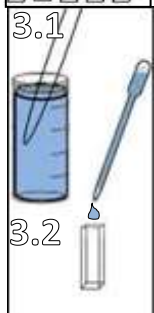
Step 2 Preparing the Reaction Cuvettes

- 1) Remove the 6 square reaction cuvettes from the foil pouch and place them in the cardboard cuvette holder, keeping the one with the blue dot separate from the others (this is your nitrate standard).
- 2) Tap cuvettes to settle contents making sure to keep them *upright* to not lose contents.
- 3) Twist off the end of the squeeze bulb with the blue dot and empty the entire contents into the cuvette with the blue dot. This is the *nitrate standard.* (2.3)
- 4) **Label the cuvette caps** with numbers, letters, or location. (1-5, A-E, etc.)
- 5) Into each of the other 5 cuvettes, empty entire contents of one buffer squeeze bulb. (2.5)
- 6) Cap all cuvettes and mix gently. Do not invert (keep liquid out of cap). Step 3 must follow within 5 minutes.



Step 3 Adding samples to Reaction Cuvettes

- 1) Within 5 minutes, pick up liquid from near the surface of your first sample with a plastic pipette (3.1).
- 2) Transfer only one drop (approximately 50 µl) of this liquid to the corresponding cuvette (Sample 1 into cuvette 1, etc.) making sure not to touch the sides or surface of the liquid with the pipette. (3.2)
- 3) Repeat for the remaining 4 samples, making sure to use a new pipette for each unique sample.
- 4) Recap the cuvettes and mix by gently shaking side to side.
- 5) Let set for **at least 10 minutes**, mixing gently every few minutes.



Step 4 Color development

- 1) Tap each snap-cap tube to a hard surface to settle color reagent powder; open tubes.
- 2) Add color reagent powder to all reaction cuvettes, including the nitrate standard. (4.1)
- 3) Firmly seal the cuvettes with square caps and vigorously mix each cuvette rapidly.
- 4) Let cuvettes develop color for about 5 minutes, mixing them several times to dissolve most of the powder. Some powder may settle to the bottom of tube; this is okay.



Step 5 Evaluating your results

After color development, compare your results within one hour using the *nitrate standard* and this chart or download NECi's free color slider application for mobile devices. The *nitrate standard* is set at 500 parts per million Nitrate-N (2200 ppm Nitrate). Contact your local crop advisor for nutrient application advice.



Nitrate-N (ppm)	0	200	500	1000
Nitrate (ppm)	0	900	2200	4400

Visit <http://nitrate.com/petiolesapnitratetesting> for more information about petiole testing

Download our free Nitrate Color Slider app available on Google Play and iTunes for easy color matching of your samples for data. Visit www.nitrate.com/mobile-apps on your mobile device's web browser, or search "Nitrate Color Slider" in the app store. Check out NECi's Handheld Photometer and app for digital data on your mobile device!