ENVIRONMENTAL EXPRESS®
a Cole-Parmer company

NECi Nitrate Kits FAQs

Why use enzymes?

Enzymes are catalysts that drive complex biological reactions. They happen to be excellent reagents for analytical chemistry because they are reliable, accurate, sensitive, selective, and non-toxic. Enzyme reactions occur in gentle, biological conditions with no organic solvents, heavy metals, high heat or pressure involved. They are sensitive, which means they have low detection limits in complex mixtures. They will ONLY react with a specific substrate, using their highly specialized binding site that only fits THEIR substrate. Once their substrate is bound, catalysis changes the substrate into a new product. After this is complete, the enzyme releases the newly formed product and is ready to begin the process again! Analyzing samples with enzymes will result in accurate and consistent data without the need for any hazardous materials or methods, unlike methods employing cadmium or zinc for nitrate analysis.

What type of products are offered?

On-Site test kits are designed for taking quick measurements of nitrate in soil, water, or plant samples where they are collected. They are simplified versions of the lab kits, providing accurate and reliable results without the need for laboratory equipment. However, there is an affordable portable, smartphone linked photometer for these kits to provide digital data in the field, increasing accuracy of the results. Laboratory test kits are for users with knowledge of basic laboratory procedures and access to laboratory equipment (test tubes, spectrophotometer, pipettes, etc.) Enzyme reagent packs are designed for experienced users that prefer to supply their own buffers and color reagents (if necessary), and are available with an optimized amount of co-factor included or as stand-alone enzyme. All reagents have been designed and developed to be used with spectrophotometers, automated liquid handling equipment, discrete analyzers, etc.

How do the kits work?

NECi's nitrate test kits use an enzyme called nitrate reductase to convert the nitrate in a sample to nitrite. Once the Griess color reagents are added, nitrite becomes visible as a pink solution. This visible pink color is analyzed by comparison to a color chart, or by measuring absorbance at 540 nm in a spectrophotometer, or NECi's handheld dual wavelength photometer. The darker the shade of pink, the more nitrate is present in the sample.

ENVIRONMENTAL EXPRESS®
a Cole-Parmer company

NECi Nitrate Kits FAQs

How do I decide which kit is right for me?

We recommend that laboratory users begin by purchasing a complete test kit for convenience of pre-made buffers and detailed instructions. This limits error and helps with troubleshooting. Once users are familiar with the assay, economical reagent packs are available which includes only the enzyme, co-factor, and enzyme reconstitution buffer. All on-site kits are simplified, pre-calculated versions of the laboratory methods and are the best choice for fast and accurate analysis anywhere on-site. These kits require no laboratory equipment or chemistry experience and can be used to analyze water, plant, soil, forage, and aqueous process effluent samples. For high throughput automated laboratory analysis, there are reagent packs complete with instructions customized to the user's equipment. These reagent packs include enzyme, co-factor, and enzyme reconstitution buffer, complete with instructions for instruments such as discrete analyzers, flow through analyzers, and more.

How does nitrate reductase work?

NECi's nitrate test kits use an enzyme (NaR) and natural reducing agent (NADH) for highly accurate and easy to use nitrate testing. The enzyme used in the nitrate test kits and reagents is a purified form of nitrate reductase (NaR). Other test kits use the toxic metals cadmium or zinc. NECi's NaR is more stable and unlike other forms of NaR as it does not require expensive NADPH as its cofactor. Instead, NADH is used.

The NaR reaction is diagrammed like this: $NADH + NITRATE \rightarrow NITRITE + NAD^{+} + OH^{-}$

NADH (nicotinamide adenine dinucleotide) is a derivative of a B vitamin. The resulting nitrite reacts with Color Reagents (the Griess Reaction) to form a pink color easily detected by eye or by a photometer at 540nm ± 10nm. In most kits, you'll end by comparing the color to that of the nitrate standards (supplied with each kit) to determine the nitrate content of your samples.

What are the sensitivity ranges of the nitrate test kits?

The user can choose to analyze nitrate in the Standard Range of 0.5 to 10 ppm nitrate-N or the Low Range of 0.01 to 1.0 ppm nitrate-N. Since NECi NTK systems all use a photometric method for evaluating the nitrate content of your samples, results are highly accurate.

ENVIRONMENTAL EXPRESS®
a Cole-Parmer company

NECi Nitrate Kits FAQs

Why use nitrate reductase to test for nitrate?

Enzymes can be trusted for clinical diagnostic tests because they're accurate, reliable, sensitive, selective, and generally environmentally benign and non-toxic. Enzymes are ideal for nitrate detection in particular because most other commercial nitrate analysis methods use a heavy metal, such as cadmium or zinc, for reducing nitrate to nitrite. These metals, especially cadmium, are toxic and so present a health risk for both the person doing the analysis and for the environment. Cadmium disposal is also difficult. NECi NTK systems use natural NaR and NADH for nitrate analysis which are both user and environmentally safe. Since NECi NTK systems are as accurate, if not more so, than other commercial systems, NaR/NADH based systems are a better choice.

What kind of samples can I test?

Users can achieve fast and accurate results from water and other aqueous samples (such as extracts of food, plants, and soil). Non-aqueous solvents interfere with the enzyme catalyzed reaction and must NOT be used. Physiological samples such as blood and plasma CAN be analyzed because generally their salt and protein content presents no significant interference. Turbid or "colored" samples can be analyzed in standard range systems but samples must be filtered and or centrifuged for low range results.

For how long are the enzyme packs stable and viable?

The enzyme packs contain the enzyme and NADH, freeze-dried in cuvettes and containers, which are then vacuum-sealed in foil pouches. There will be a 6-month expiration date on all kits, but packs consistently are stable for more than 6 months stored at any temperature below 22°C; stability study samples have been effective even after multiple years. The enzyme can remain stable for years if stored in these packets at -20°C.

For how long is the NADH in kits stable and viable?

Because NADH oxidizes to NAD+ over time it will eventually be unable to supply the electrons needed for the nitrate reduction step. Use these precautions for NADH: store frozen for one to two weeks, or portioned into small aliquots - the volume you would use in a day's work. Store the aliquots in a fridge (at least one week) or frozen (one month). You may see longer activity retention - a month or more - but one or two weeks is safe.

ENVIRONMENTAL EXPRESS®
a Cole-Parmer company

NECi Nitrate Kits FAQs

For how long is the assay buffer stable?
The assay buffer, when stored cold, is stable for one month.

For how long are the color reagents stable?
Both Color Reagent 1 and Color Reagent 2 are stable enough for use after being stored for up to one month cold, at or below 4°C, and dark. Color reagent 2 (NED reagent) will start to turn brownish after some weeks after the addition of water. This will cause a loss in sensitivity as the background in blanks becomes too high.

Once the enzyme pack (foil pouch with cuvettes) is open, for how long is the enzyme good?
There will be a 6-month expiration date on all kits. Packs consistently are stable for more than 6 months stored at any temperature below 22°C; stability study samples have been effective even after multiple years. The enzyme can be stable for years if stored in these packets at -20°C.

For how long are the prepared solutions stable and viable?
Once the kit process has been started you should aim to complete it in a timely fashion, being sure to FOLLOW STATED TIMES carefully. The most common errors are either stopping the reduction reaction too soon, or not proceeding to the next step in time. DO NOT let the solutions you prepare sit for extended periods of time. Once started, the kit process should be completed that day.

What if there is not pink in one sample or standard?
If you had reason to expect nitrate in this sample, the most likely cause is you left out one of the reagents. Each tube must get: buffer, NADH, and NaR (enzyme) and then the two color reagents. Exclusion of buffer or color reagents will result in that sample having a significantly smaller volume, while if NADH or NaR are left out the volume will look the same. Repeat the analysis of the sample or standard being sure to get every component into the tube.

What if all samples and standards have a dark pink color, including the reagent blank?
Most probably, there is nitrate or nitrite in your deionized water. Therefore, you should obtain new deionized water.

ENVIRONMENTAL EXPRESS®
a Cole-Parmer company

NECi Nitrate Kits FAQs

What if the pink of the nitrate standard is not dark enough or reading "low" with my photometer?

If this problem of light coloration is occurring, you might have stopped the reaction too soon by preemptively adding the Color Reagent. Allow enough time for the reactions to occur and carefully follow the time-frames outlined in the instructions. For Simple NTKs: Be sure to wait AT LEAST 10 MINUTES AFTER adding samples to the reaction/standard cuvettes BEFORE adding the Color Reagent. If necessary, adding more time DURING the reaction step, BEFORE the color reagent is added will not negatively affect results.

What if the samples are higher absorbance (pink is darker) than the highest standard?

The sample has too much nitrate and must be diluted and reanalyzed. Dilute the sample with nitrate-free deionized water 1:4 (five-fold dilution) and 1:9 (ten-fold dilution) and analyze the nitrate again. If the sample is still off the standard curve (ie. higher in absorbance than the highest standard), then you will have to dilute it more, even up to 50- or 100-fold.

How important is it to follow the times outlined in the instructions?

Following the stated times is VERY IMPORTANT. Be especially sure to give the enzyme reaction time to fully complete: not allowing the reaction time to finish is the most common user mistake.

Do I have to blank the photometer each time I use it?

You must blank the NECi Photometer each time you turn on the device.

The app is informing me that the Bluetooth connection has failed, what can I do to fix this?

Ensure that the photometer is properly paired with your android device before you begin using the NECi Photometer app. Navigate to your Android device's Bluetooth settings, and make sure that your photometer is paired. If it is paired and you are still seeing connection failures, unpair your photometer and re-pair it within the Bluetooth settings.

Why won't my photometer show up in the "Selected Photometer" list?

First, make sure that your photometer is paired to your Android device. If it is, exit the "Selected Photometer" list, and select it again, your device should now show up.

NECi Nitrate Kits FAQs

Why is my photometer showing incorrect results for my standard cuvette?
The NECi Photometer and NECi Easy Test Kits have a combined margin of error of less than or equal to 10%.

Why is my photometer responding slowly/giving consistently incorrect results?
Ensure that the battery is sufficiently charged. A full charge is indicated by the top LED on the front of the photometer. When plugged into a charger, the LED will appear green when fully charged, and red when not fully charged. If the battery is sufficiently charged and the problems are still occurring, contact customer service.

The app is informing me that the Bluetooth connection has failed, what can I do to fix this?
Ensure that the photometer is properly paired with your android device before you begin using the NECi Photometer app. Navigate to your Android device's Bluetooth settings, and make sure that your photometer is paired. If it is paired and you are still seeing connection failures, unpair your photometer and re-pair it within the Bluetooth settings.