SKU: NTK-ND-25

Nitrate by NADH Disappearance: Lab Kit for Food Testing

(0 to 10 ppm Nitrate-N)

Accurate, economical, and safe nitrate analysis.

- Analyze 25 samples
- Nitrate Standards included with the kit

Nitrate Units	US EPA	CA & Europe	Molarity
Standard Range Kit	**0.5 - 10.0 ppm Nitrate-N**	**2.0 - 44 ppm Nitrate**	**36 - 714 µM Nitrate**

Nitrate is reported in different units depending on your field of use and where you live.

OVERVIEW

- ✓ **Store kit refrigerated** or below 60°F (15°C).
- ✓ *See box for expiration date.*

This kit will provide reliable estimates of nitrate content when used as supplied.

For **quantitative data**, measure reagents using pipets and read assay results with a spectrophotometer at 340nm.

♦ EQUIPMENT AND REAGENTS

You will need to supply:

- ❑ distilled or deionized water
- ❑ clean, nitrate-free containers if you are collecting samples to analyze later

We use the abbreviation "d-I water" for distilled or deionized water.

NECi Superior Enzymes
334 Hecla Street
Lake Linden, Michigan 49945
Tech: 906.296.1130
Sales: 906.296.1115

This Nitrate test kit is based on the enzyme Nitrate Reductase (NaR), catalyzing the reduction of Nitrate to Nitrite using the natural electron donor NADH. The concentration of Nitrate in the original sample is determined by measuring absorbance versus Nitrate concentration in Nitrate Standards. Nitrate can be determined in water samples and extracts of plant tissues, soils and foods. The test is designed to measure Nitrate in the range of 0.5 to 10 ppm Nitrate-N in up to 25 samples or standards. The Nitrate concentration can also be expressed as µM Nitrate, where range is 36 to 714 µM Nitrate, or ppm Nitrate, where range is 2.0 to 44 ppm Nitrate.

Prepare your food samples according to your standard procedures. Make sure your samples are ready to assay before starting the procedure on page 3.

Supplied in NECi Test Kit

- ❑ **Assay Buffer (AB)** in liquid form – two 50 ml tubes
- ❑ **NADH** in freeze-dried form - two tubes in amber bag
- ❑ **Nitrate Reductase (NaR)** in freeze-dried form – one tube in foil pouch
- ❑ **Enzyme Diluent** – one squeeze-bulb
- ❑ **Nitrate Standard (100 ppm Nitrate-N) -** in liquid form – one 1.5 ml tube
- ❑ **Microcentrifuge tubes –** six tubes for preparing Nitrate Standards
- ❑ **Cuvettes -** 7 for reactions, UV compatible

Supplied by User

- ❑ **10 ml graduated cylinder.**
- ❑ **Variable pipetters** (10 to 100 µl and 100 to 1000µl).
- ❑ **Spectrophotometer** capable of reading at 340 nm± 20 nm, with a glass or plastic cuvette (approx. volume 3.5 ml).
- ❑ **Timer** (0 to 20 minutes) – a clock or stop watch is adequate.
- ❑ **Deionized or distilled water** (d-I water; must be "Nitrate-free" to avoid high background).
- ❑ **Ice and Ice Bucket.**
- ❑ Extra cuvettes may be needed. Make sure they are UV compatible (optical glass, plastic or quartz)

NEED HELP? Contact NECi
Toll Free: 1-888-NITRATE FAX: 1-906-296-8003
Email: tech@nitrate.com
Visit us on the web: www.nitrate.com

♦ REAGENT PREPARATION

Step 1 **Assay Buffer** – ready to use from kit. Warm to room temperature for nitrate tests. If desired, the assay buffer may be more quickly warmed in a 30ºC water bath.

Step 2 Remove a tube of **NADH** from amber bag, tap tube to settle contents, add 1.5 ml **d-I water** and replace cap. Mix by inversion several times. Keep on ice during use.

Step 3 Remove **NaR** vial from foil pouch and tap tube to settle contents before opening. Twist off the end of the **Enzyme Diluent Squeeze Bulb** and completely empty the contents into the **NaR** vial. Replace the cap and mix by inversion 3 times. Allow to stand at room temperature for at least 10 minutes, with mixing at 5 and 10 minutes. Then keep on ice during use.

NOTES ON THE REAGENTS

- **Assay Buffer** – 28 mM KH_2PO_4, 0.025 mM EDTA; pH 7.5
- **NADH** – 2.4 mg per tube.
- **Nitrate Reductase (NaR)** – 1.0 unit of NaR per tube.
- **Nitrate Standard** – 1 vial of 100 ppm nitrate-N.

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♦ STANDARD PREPARATION

- Transfer **1 ml of 100 ppm Nitrate-N Standard** into a test tube containing **9 ml d-I water** to make a **10 ppm Nitrate-N Standard.** Use the 6 microtubes (provided in kit) to prepare Nitrate Standards as shown in table below. Cap and mix the tubes by inversion before use.

Vol 10 ppm Nitrate-N Standard	Volume d-I water	Resulting Standard (ppm Nitrate-N)	Resulting Standard (ppm Nitrate)	Resulting Standard (µM)
1000	0	10.0	44	712
750	250	7.5	33	534
500	500	5.0	22	356
250	750	2.5	11	178
100	900	1.0	4.4	71.2
50	950	0.5	2.2	35.6

♦ NITRATE ASSAY PROCEDURE

- The following procedure is written for single determinations.
- For greater accuracy, replicates can be run.

WASTE DISPOSAL

Follow all local guidelines and regulations. If there are no local guidelines, wash the waste down the sink with large amounts of running water.

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♦ CALCULATIONS

STEP 1 Pipette **100 µl d-I water** into one cuvette for use as reagent blank.

STEP 2 Pipette **100 µl** of your prepared **samples** and **standards** into the required number of cuvettes.

STEP 3 Add **1.80 ml Assay Buffer** to each cuvette.

STEP 4 Add **100 µl NADH solution** to each cuvette. Cap and mix thoroughly.

STEP 5 Zero the spectrophotometer with d-I water in a cuvette. Read absorbance of each cuvette at 340nm (A1).

STEP 6 To start the reaction add **40 µl NaR** solution to each tube. Recap and mix thoroughly.

STEP 7 Read A340 of each cuvette at exactly 20 minutes (A2) after adding NaR.

STEP 8 Read A340 of each cuvette at exactly 30 minutes (A3).

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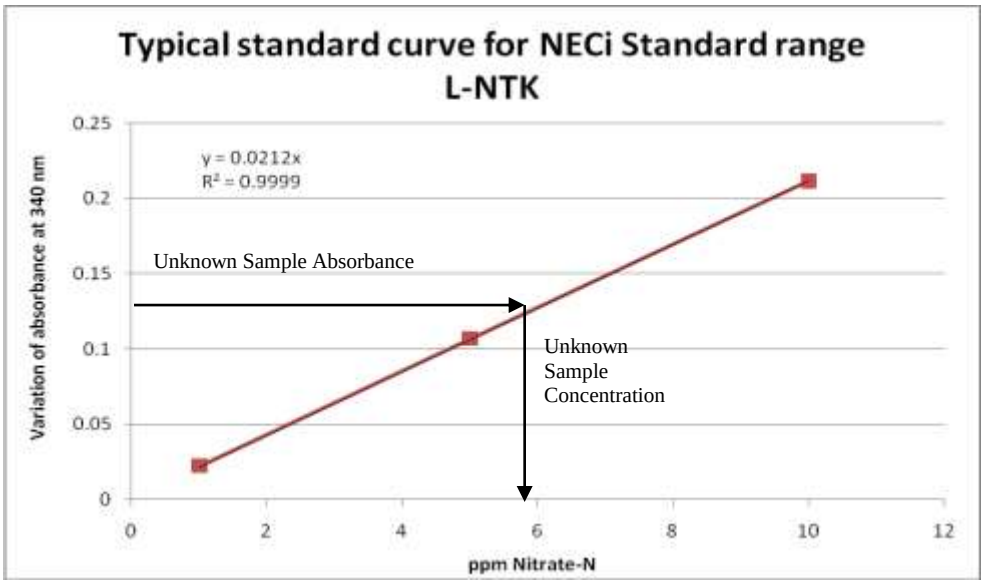
STEP 1 Determine absorbance differences (A1-A2) and (A2-A3) for the blank and samples.
Absorbance difference of the blank $= (A1\text{-}A2)_{blank} - 2 \times (A2\text{-}A3)_{blank}$
Absorbance difference of the sample $= (A1\text{-}A2)_{sample} - 2 \times (A2\text{-}A3)_{sample}$
$\Delta A_{nitrate}$ = absorbance difference $_{sample}$ – absorbance difference $_{blank}$

STEP 2 Generate a standard curve for the Nitrate Standard (see example below). Using linear graph paper or a computer plotting program such as Sigma Plot® or spreadsheet such as Excel®, plot the ppm Nitrate-N on the x-axis, and the ΔA-340 nm for each nitrate standard on the y-axis. If plotting by hand, draw a straight line through the points for the Nitrate Standards. If plotting by computer, the slope of the line can be calculated for determining Nitrate-N ppm in the unknown samples.

STEP 3 Using the standard curve, determine the ppm Nitrate-N for the sample: (a) Find the ΔA-340 nm for the sample on the y-axis of the standard curve. (b) Follow over along a horizontal line to where the line intersects the standard curve. Trace down the x-axis and read the ppm of Nitrate-N on the x-axis.



UNKNOWN SAMPLES WITH HIGH NITRATE

This NECi Nitrate Test Kit is capable of determining Nitrate levels of up to 10 ppm Nitrate-N (714 µM Nitrate). If an unknown sample is found to have more than 10 ppm Nitrate-N, the sample may be diluted with d-I water 1:10 to allow an exact determination. For example, take 100 µl of sample and add 900 µl of deionized water to make a 1:10 dilution and then assay 100 µl of the diluted sample. After finding the Nitrate content of the diluted sample, multiply the Nitrate concentration by 10 to find the Nitrate concentration in the original sample . NOTE: Keep the sample volume constant by diluting the sample rather than using a smaller volume of sample in the assay.

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DETERMINING NITRATE IN MOLAR UNITS

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Use a conversion of 1ppm Nitrate-N = 71 µM Nitrate. One ppm Nitrate-N = 1 mg of Nitrogen/liter. Since Nitrogen has a molecular weight of 14 g/mole, then the molar concentration is:

$$(0.001\ g/1) \div (14\ g/mole) = 0.000071\ M\ Nitrogen =$$
$$0.000071\ M\ Nitrate = 71\ \mu M\ Nitrate$$

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DETERMINING NITRATE AS A QUANTITY

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(Using the 100 µl Sample Size). In the tube where the 10 ppm nitrate-N standard is determined, there is approx. 71 nmol of Nitrate [(714 nmol Nitrate/ml) x (0.10 ml) = 71.4 nmol]. So the example standard curve would have a slope of 0.0003 A-340 nm/nmol Nitrate (calculated from slope = 0.0212 A-340 nm/71.4 nmol Nitrate).

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FOODSAMPLE PREPARATION

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Use your standard food sample preparation method prior to starting the nitrate assay procedure on page 3.