SKU: NTK-MPSR

Standard Range Microplate Nitrate Test Kit

(0.5 to 10 ppm Nitrate-N)

Accurate, economical, and safe nitrate analysis.

- Analyze 96 samples (including standards)
- 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 colorimeter at 540nm.

♦ 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.

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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 Nitrite reacts with color reagents (dyes) under acidic conditions to produce a visible color. All reaction steps occur in the microplate. The concentration of Nitrate in the original sample is determined by measuring absorbance with a microplate reader using a 540 nm filter (or a filter as close to 540 nm as possible), 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 using a 96-well microplate. 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 44ppm Nitrate. Nitrite can also be determined by omitting NaR and NADH from the test (see Determining Nitrite, page 4).

If testing seawater, salt water or brackish water, follow the blue high-lighted instructions.

Chloride is a mild inhibitor of NaR. The color development when analyzing seawater is not as intense as other types of samples. Nitrate Standards prepared in saltwater solves the problem.

Supplied in NECi Test Kit

- ❑ **Assay Buffer** in liquid form - one 15 ml amber bottle
- ❑ **Color Reagent No. 1** in solid form - one 15 ml amber bottle
- ❑ **Color Reagent No. 2** in solid form - one 15 ml amber bottle
- ❑ **NADH** in freeze-dried form - one tube 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
- ❑ **Microplate** - one 96-well microplate with flat bottom wells
- ❑ **Pipetter Reservoir Basins** - two clear plastic containers for reagents
- ❑ **Microcentrifuge tubes -** six tubes for preparing Nitrate Standards
- ❑ **Salt Water** - one 20 ml tube, green cap, only if you are testing seawater

Supplied by User

- ❑ **25 ml graduated cylinder**
- ❑ **Variable pipetters -**10 to 100 µl and 1ml, or multi-well pipetter
- ❑ **Vortex-type mixer**
- ❑ **Microplate mixer**
- ❑ **Microplate reader** capable of reading at 540 nm± 20 nm
- ❑ **Several test tubes** (Clean and Nitrate-free)
- ❑ **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)
- ❑ **2.5 ml of concentrated HCl**
- ❑ **Ice and Ice Bucket**

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 **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.

Step 3 Prepare **3 N HCl** by adding **2.5 ml concentrated HCl** to **7.5 ml d-I water.** Mix.

Step 4 Add **10 ml 3 N HCl** to **Color Reagent No. 1** bottle. Mix by shaking well.

Step 5 Add **10 ml d-I water** to **Color Reagent No. 2** bottle. Mix by shaking well.

Step 6 Remove 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 until use.

Step 7 In pipetter reservoir basin (included in kit), add **9 ml Assay Buffer, 1 ml NaR solution** (prepared in Step 2), and **0.5 ml NADH solution** (prepared in Step 6). Mix thoroughly. **Note**: this step should be done just prior to use in Step 3 of the Nitrate Assay Procedure.

NOTES ON THE REAGENTS

- **Assay Buffer** - 28 mM KH_2PO_4, 0.025 mM EDTA; pH 7.5
- **Color Reagent No. 1** - 1% Sulfanilamide in 3N HCl
- **Color Reagent No. 2** - 0.02% N-Naphthylethylenediamine in d-I water.
- **NADH** – 2.4 mg NADH
- **Nitrate Reductase (NaR)** – 1.0 unit of NaR per tube
- **Nitrate Standard** – 1 vial of 100 ppm nitrate-N
- **Salt Water** - may contain 0.05 ppm nitrate-N, giving your blank a slight pink color

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♦ STANDARD PREPARATION

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- 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. **If you are testing seawater**, use the **Salt Water** provided instead of d-I water to prepare standards.

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	714
750	250	7.5	33	534
500	500	5.0	22	356
250	750	2.5	11	178
100	900	1.0	5.5	71.2
50	950	0.5	2.2	35.6

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♦ NITRATE ASSAY PROCEDURE

- The following procedure is written for 2 replicates of each standard, unknown sample and reagent blank.

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.

Using the **Microplate Sample Template**, assign and record a set of 2 wells for ***each*** **Nitrate Standard, Sample and Reagent Blanks.**

STEP 1 Pipette **10 µl d-I water** into 2 wells for reagent blanks. If testing seawater, use the **Salt Water** provided instead of d-I water.

STEP 2 Pipette **10 µl** of the **standards** and **samples** into designated wells.

STEP 3 Add **90 µl NaR-Assay Buffer-NADH solution** (prepared in Step 7 of Reagent Preparation) to each well. Shake on a plate mixer for ~ 20 minutes @ 800 rpm.

STEP 4 If using a multi-pipetter, rinse the pipetter reservoir basins with **d-I water**. Transfer **Color Reagent No. 1** to one basin and **Color Reagent No. 2** solution to the other basin.

STEP 5 Add **50 µl Color Reagent No. 1** and **50µl Color Reagent No. 2** solution to each well. Shake on a plate mixer for ~ 10 minutes @ 800 rpm.

STEP 6 **Zero the plate reader with a Reagent Blank well** using a 540 nm filter (or a filter within 20 nm or 540 nm).

STEP 7 **Read absorbance of all wells.** Transfer results to your computer system for analysis and printing.

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♦ CALCULATIONS

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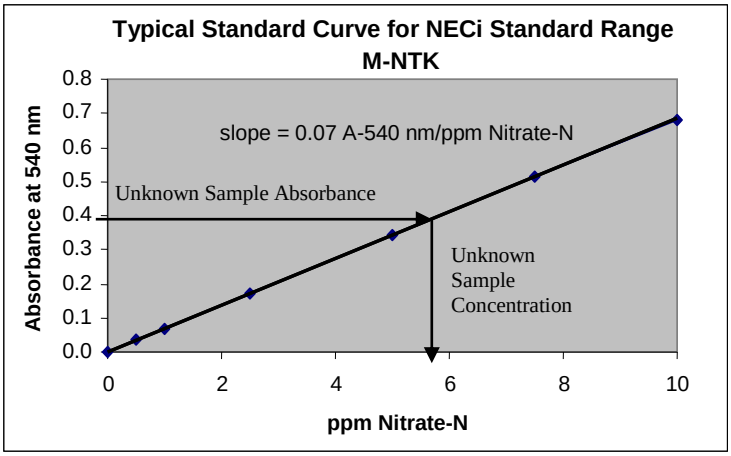
STEP 1 To correct for any background absorbance due to the reagents, subtract the mean absorbance of the reagent blanks from the mean absorbance of each nitrate standard and unknown sample:

Corrected mean sample A-540 nm = (mean A-540 nm for samples) – (mean A-540 nm for reagent 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 A-540 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 corrected A-540 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.



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UNKNOWN SAMPLES WITH HIGH NITRATE

This NECi Nitrate Test Kit is capable of determining Nitrate levels of up to 10 ppm Nitrate-N (44 ppm Nitrate or 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 10 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

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:

$$(0.001 \text{ g}/1) \div (14 \text{ g/mole}) = 0.000071 \text{ M Nitrogen} = 0.000071 \text{ M Nitrate} = 71 \text{ µM Nitrate}$$

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DETERMINING NITRATE AS A QUANTITY

(Using the 10 µl Sample Size). In the wells where the 10 ppm nitrate-N standard is determined, there is approx. 7.14 nmol of Nitrate [(714 nmol Nitrate/ml) x (0.01 ml) = 7.14 nmol]. So the example standard curve would have a slope of 0.010 A-540 nm/nmol Nitrate (calculated from slope = 0.07 A-540 nm/7.14 nmol Nitrate).

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NITRATE DETERMINATION IN PLANT LEAF EXTRACTS

To determine how much Nitrate is in one gram of leaf tissue, grind the tissue in 10 ml d-I water and measure the total volume of extract after the solids are filtered off. Take 10 µl of extract for the Nitrate assay, conduct the Nitrate Test Kit assay and find the amount of Nitrate present in nmoles. Determine the total amount of Nitrate in the extract [= (total volume of extract) x (nmol Nitrate/10 µl of extract)]. Divide this total amount of Nitrate by the weight of plant tissue to find the amount of Nitrate per unit of tissue (grams in this example). The green or brown color of the leaf extract does not significantly interfere with Nitrate determinations since the plant extract is diluted 20-fold in the assay. The most quantitative analysis of leaf Nitrate content is obtained when the leaves are boiled for 20 min. After boiling, cool on ice and then filter the sample to recover aqueous extract. Finally, make the volume back up to 10 ml to compensate for water lost during boiling. ***Ask NECi for a detailed protocol for extracting either fresh plant leaves or dried leaf material for Nitrate Assays.***

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Determining Nitrite

Nitrite can be determined by omitting NaR and NADH from the samples. Replace the solution in Step 3 of the Nitrate Assay Procedure with 90 µl of d-I water and eliminate Step 4. Prepare Nitrate standards as described in the normal Nitrate Assay Procedure with both NADH and NaR added and use the Nitrate Standard Curve for estimating Nitrite content.

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Notes on Nitrate in Water

The Clean Water and Safe Drinking Water Acts (U.S. EPA 1974) set Maximum Contaminant Level (MCL) for potable water at 10 ppm Nitrate-N (10 mg Nitrate-N per liter). California and European standards are 45 ppm nitrate (45 mg nitrate per liter). If you find drinking water with 7 to 10 ppm Nitrate-N or more, advise users to seek a professional test of their water. Environmental water samples usually contain 1 to 2 ppm Nitrate-N or less.