



The Nitrate Elimination Co., Inc.
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NECi Sample Preparation Protocol for Nitrate Analysis in SOIL SAMPLES

LOW RANGE (page 1) AND STANDARD RANGE (page 2)

Using NECi L-NTK-202 or 204 *Low Range* Test Tube Kit

Or NECi M-NTK-302 *Low Range* Microplate Kit

****** Low Range: 0.05 - 1.0 ppm Nitrate-N [0.25 - 5.0 ppm nitrate or 3.5 - 70 μ M nitrate] ******

Protocol I: Screening Method

Prepare extracts of soil samples while FD-NaR is being reconstituted (per kit instructions).

1. Place 1/4 teaspoon soil sample into a 15 ml tube.
2. Add 10 ml deionized or distilled (de-I) water.
3. Cap tube and shake ~1 min
4. Allow to settle for 10 min.
5. Repeat steps 3 & 4.
6. Use this extract for assay.
7. If results are > 1.0 ppm, dilute extract 1:10 and 1: 100 with de-I water and repeat assay.

Protocol II: Semi-Quantitative Method

Prepare extracts of soil samples while FD-NaR is being reconstituted (per kit instructions).

1. Place 1 gram of soil sample into a 15 ml tube.
2. Add 10 ml de-I water.
3. Cap tube and shake ~1 min.
4. Allow to settle for 10 min.
5. Repeat steps 3 & 4. Filter sample if desired.
6. Use 500 μ l (for L-NTK) or 50 μ l (for M-NTK) of this extract for assay.
7. If results are > 1.0 ppm, dilute extract 1:10 and 1:100 and repeat assay.

Protocol III: Quantitative Method *Note: See Instructions for drying samples to constant weight.*

As Protocol II, except:

1. Use pipet for sample addition (500 μ l or 50 μ l).
2. Prepare nitrate standard curve using supplied nitrate standards. Read absorbance of standards at A540 \pm 20nm.
3. Read sample absorbance. Calculate nitrate content from the Standard Curve results. Calculation instructions are included with the kits.

Our studies indicate that most of the nitrate is recovered from soil samples with water only. However, if you are already using a soil extraction method with 2M KCl, etc., you can still use NECi's NTK's. Simply make the nitrate standard solutions *in your extraction solution* instead of water, and then use these standards as directed in the kit instructions. This will compensate for any interference that may be caused by your extraction solution.

Questions? Problems? Ready to order? Call us at 888/NITRATE!



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NECi Sample Preparation PROTOCOL FOR NITRATE ANALYSIS IN SOIL SAMPLES

Using NECi L-NTK-201 or 203 *Standard Range* Test Tube Kit

Or NECi M-NTK-301 *Standard Range* Microplate Kit

****Standard Range: 0.5 - 10.0 ppm Nitrate-N [2.5 - 45 ppm nitrate or 35 - 710 µM nitrate]****

Protocol I: Screening Method

Prepare extracts of soil samples while FD-NaR is being reconstituted (per kit instructions).

8. Place 1/4 teaspoon soil sample into a 15 ml tube.
9. Add 10 ml deionized or distilled (de-I) water.
10. Cap tube and shake ~1 min
11. Allow to settle for 10 min.
12. Repeat steps 3 & 4.
13. Use this extract for assay.
14. If results are > 10 ppm, dilute extract 1:10 and 1: 100 with de-I water and repeat assay.

Protocol II: Semi-Quantitative Method

Prepare extracts of soil samples while FD-NaR is being reconstituted (per kit instructions).

8. Place 1 gram of soil sample into a 15 ml tube.
9. Add 10 ml de-I water.
10. Cap tube and shake ~1 min.
11. Allow to settle for 10 min.
12. Repeat steps 3 & 4. Filter sample if desired.
13. Use 50 µl (for L-NTK) or 10 µl (for M-NTK) of this extract for assay.
14. If results are > 10 ppm, dilute extract 1:10 and 1:100 and repeat assay.

Protocol III: Quantitative Method *Note: See Instructions for drying samples to constant weight*

As Protocol II, except:

4. Use pipet for sample addition (50µl or 10 µl).
5. Prepare nitrate standard curve using supplied nitrate standards. Read absorbance of standards using a colorimeter at $A_{540} \pm 20\text{nm}$.
6. Read sample absorbance. Calculate nitrate content from the Standard Curve results.

Our studies indicate that most of the nitrate is recovered from soil samples with water only.

However, if you are already using a soil extraction method with 2M KCl, etc., you can still use NECi's NTK's. Simply make the nitrate standard solutions *in your extraction solution* instead of water, and then use these standards as directed in the kit instructions. This will compensate for any interference that may be caused by your extraction solution.

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