

# Nitrate in Soil Samples

Reflectometric determination after reduction to nitrite and reaction with Griess reagent

## Introduction

Nitrate ( $\text{NO}_3^-$ ) is a major form of inorganic nitrogen in agricultural soils and a primary nitrogen source for plant uptake. Its concentration is governed by fertilizer application, organic matter mineralization, climatic conditions, and crop management practices.<sup>1,2</sup> Due to its high solubility and mobility in the soil solution, nitrate readily leaches through the soil profile into groundwater and surface water, posing a risk recognized across agricultural systems worldwide.<sup>1,2</sup> Accurate determination of soil nitrate is therefore essential for optimizing fertilizer use, improving nitrogen-use efficiency, and mitigating environmental losses.<sup>2</sup>

For analysis, nitrate is first extracted from soil using a calcium chloride ( $\text{CaCl}_2$ ) solution, which provides stable ionic conditions and ensures the recovery of readily soluble, plant-available nitrogen forms.<sup>3</sup> The resulting extract can then be quantified using a range of analytical techniques, including colorimetric, reflectometric, or photometric methods, depending on the available instrumentation and required throughput.

## Experimental

The procedure detailed in this application note encompasses sample preparation and analysis using the Reflectoquant® Nitrate Test strips in conjunction with the portable RQflex® 20 reflectometer. This method offers a rapid, cost-effective, and reliable approach to assessing Nitrate concentrations while also allowing comparisons with standard method (photometry) to ensure the accuracy and validity of results.

## Method

Nitrate ions are reduced to nitrite ions by a reducing agent. In the presence of an acidic buffer, these nitrite ions react with an aromatic amine to form a diazonium salt, which in turn reacts with *N*-(1-naphthyl)-ethylenediamine to form a red-violet azo dye that is determined reflectometrically.

## Measuring Range

|                                       |                             |
|---------------------------------------|-----------------------------|
| Reflectoquant® Nitrate Test (1.16995) | 3–90 mg/L $\text{NO}_3^-$   |
|                                       | 3–90 mg/kg $\text{NO}_3^-$  |
| Reflectoquant® Nitrate Test (1.16971) | 5–225 mg/L $\text{NO}_3^-$  |
|                                       | 5–225 mg/kg $\text{NO}_3^-$ |

## Applicable Sample

Soil samples

## Influence of Foreign Substances

Foreign substances in the sample solution can

- Increase the measurement value because of an amplification of the reaction
- Lower the measurement value because of a prevention of the reaction

A quantification of these effects is stated in tabular form in the respective package insert for the most important foreign ions and substances. The tolerance limits have been determined for the individual ions and substances; they may not be evaluated cumulatively.

In the case of samples with a complex, in many cases inexact known composition (matrix) it is particularly difficult to estimate the potential influence of the foreign substances on the analysis (matrix effect). The following instructions describe a method by means of which the user can test whether a matrix effect is present or not.

- Interference check by dilution
- Interference check by spiking

## Reagents, Instruments and Materials

### Test/Reagent Kit(s)

- Reflectoquant® Nitrate Test, 3–90 mg/L NO<sub>3</sub><sup>-</sup> (**1.16995**)
- Reflectoquant® Nitrate Test, 5–225 mg/L NO<sub>3</sub><sup>-</sup> (**1.16971**)

### Instrument(s) & Devices

- Reflectometer RQflex® 20, Reflectoquant® (**1.17246**)

### Other Reagents and Accessories

- Calcium chloride dihydrate for analysis EMSURE® ACS, Reag. Ph Eur (**1.02382**)
- MQuant® Nitrate Test, 10–500 mg/L NO<sub>3</sub><sup>-</sup> (2.3–113 mg/L NO<sub>3</sub><sup>-</sup>-N) (**1.10020**)
- Distilled water (e.g., from a suitable Milli-Q® system) or water for analysis (**1.16754**)
- Balance
- Volumetric flask
- Nitrate-free folded Filter

### Products for AQA

- RQcheck set for RQflex® 20 Reflectometer (**1.17247**)
- Recalibration Set for RQflex® 20 Reflectometer (**1.16954**)
- Nitrate standard solution CRM 1000 mg/L (NO<sub>3</sub>), traceable to SRM from NIST NaNO<sub>3</sub> in H<sub>2</sub>O Certipur® (**1.04613**)

## Analytical Procedure

### Sample Preparation

#### Preparation of the extraction solution (CaCl<sub>2</sub> solution 0.01 mol/L):

- Dissolve 1.47 g calcium chloride dihydrate (**1.02382**) in distilled water and make up to 1,000 mL with distilled water in a volumetric flask.

#### Preparation of the sample solution:

- Homogenize ca. 100 g (exactly weighed) of soil sample with 100 mL of 0.01 mol/L CaCl<sub>2</sub> solution by shaking for 30 minutes.
- Filter through nitrate-free folded filter
- If applicable, check the nitrate content with the MQuant® Nitrate Test to assess the expected target value.

## Measurement

- Press the START button of the reflectometer and - this is imperative - at the same time immerse both reaction zones of the test strip in the pretreated sample (15–30 °C) for 2 seconds.

- Carefully allow excess liquid to run off via the long edge of the strip onto an absorbent paper towel for 1 second.
- Approx. 10 seconds before the end of the reaction time, insert the strip all the way into the strip adapter with the reaction zones facing the display.
- After the end of the reaction time (60 s), read off the result from the display in mg/L NO<sub>3</sub><sup>-</sup>. The result is automatically stored.

### Notes on the measurement

For detailed operating procedures, consult the operating instructions for RQflex® instrument and the package insert of the Reflectoquant® Nitrate Tests.

## Analytical Quality Assurance

Analytical quality assurance (AQA) is recommended before each measurement series. Check the instrument using the RQcheck. If RQcheck failed, perform a recalibration using the recalibration set and repeat the RQcheck. For more details see RQflex® 20 manual.

To check test strips, measurement device, and handling (recommended before each measurement series):

Dilute the nitrate standard solution with distilled water to 30 mg/L NO<sub>3</sub><sup>-</sup> (**1.16995**) or 50 mg/L NO<sub>3</sub><sup>-</sup> (**1.16971**) and analyze as described in the section measurement.

## Calculation

$$\text{Nitrate content [mg/kg]} = \frac{\text{Measured value [mg/L]} \times \text{Vol. CaCl}_2 \text{ sol. [mL]}}{\text{Weight of sample [g]}}$$

## Conversion to kg Nitrate-N/hectare (layer of soil)

$$\text{kg Nitrate-N/ha} = A \times \text{BF} \times 3 \times D \times 0.226$$

where:

A = Measured value

BF = Factor for wetness of soil and extraction BF = 1.41 at an extraction ratio of 1 + 1 and 83% dry mass

3 = For a 30 cm thick layer of soil

D = Soil density (1.5 kg/dm<sup>3</sup>)

0.226 = Conversion factor NO<sub>3</sub><sup>-</sup> into NO<sub>3</sub>-N

## Results

Here in this study, twenty-six different soil samples were used as test samples for measurement and comparison.

The results were verified using photometric standard analysis as orthogonal method to Reflectoquant® test kit.

**Table 1.** Comparison of Reflectoquant® Nitrate Test and standard method (photometry)

| Sample | Reflectoquant® method | Standard method   |
|--------|-----------------------|-------------------|
|        | [kg Nitrate-N/ha]     | [kg Nitrate-N/ha] |
| 1      | 92                    | 97                |
| 2      | 89                    | 93                |
| 3      | 86                    | 94                |
| 4      | 100                   | 99                |
| 5      | 46                    | 44                |
| 6      | 34                    | 33                |
| 7      | 48                    | 45                |
| 8      | 46                    | 43                |
| 9      | 31                    | 28                |
| 10     | 32                    | 28                |
| 11     | 36                    | 32                |
| 12     | 30                    | 33                |
| 13     | 39                    | 39                |
| 14     | 76                    | 77                |
| 15     | 44                    | 42                |
| 16     | 61                    | 63                |
| 17     | 39                    | 37                |
| 18     | 66                    | 68                |
| 19     | 44                    | 42                |
| 20     | 68                    | 69                |
| 21     | 48                    | 45                |
| 22     | 87                    | 84                |
| 23     | 46                    | 45                |
| 24     | 53                    | 51                |
| 25     | 116                   | 110               |
| 26     | 111                   | 110               |

## Conclusion

The Reflectoquant® Nitrate Test, used in conjunction with the RQflex® 20 reflectometer, offers farmers a rapid, cost-effective, and reliable analytical approach for routine nitrate monitoring. Unlike traditional laboratory methods, reflectometric analysis enables on-site measurements, facilitating real-time quality control decisions.

As shown in this application note, the rapid test has a mean absolute deviation of 5% versus the reference method. Validation using orthogonal approaches, such as photometric analysis, demonstrates that its results are comparable to those of traditional techniques. This combination of efficiency and accuracy makes the Reflectoquant® method a valuable tool for analyzing nitrate levels in soil as a screening method.

## References

1. Fan, J.; Hao, M.; Malhi, S. S. Accumulation of Nitrate-N in the Soil Profile and Its Implications for the Environment under Dryland Agriculture in Northern China: A Review. *Can. J. Soil Sci.* **2010**, *90* (3), 429–440. DOI: [10.4141/CJSS09105](https://doi.org/10.4141/CJSS09105).
2. Cameron, K. C.; Di, H. J.; Moir, J. L. Nitrogen Losses from the Soil/Plant System: A Review. *Ann. Appl. Biol.* **2013**, *162* (2), 145–173. DOI: [10.1111/aab.12014](https://doi.org/10.1111/aab.12014).
3. Houba, V. J. G.; Temminghoff, E. J. M.; Gaikhorst, G. A.; van Vark, W. Soil Analysis Procedures Using 0.01 M Calcium Chloride as Extraction Reagent. *Commun. Soil Sci. Plant Anal.* **2000**, *31* (9–10), 1299–1396. DOI: [10.1080/00103620009370514](https://doi.org/10.1080/00103620009370514).

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## Featured Products

| Description                                                                                                                            | Cat. No.       |
|----------------------------------------------------------------------------------------------------------------------------------------|----------------|
| <b>Reflectoquant® Instruments and Strips</b>                                                                                           |                |
| Reflectometer RQflex® 20, Reflectoquant®                                                                                               | <b>1.17246</b> |
| Reflectoquant® Nitrate Test, 3–90 mg/L Nitrate                                                                                         | <b>1.16995</b> |
| Reflectoquant® Nitrate Test, 5–225 mg/L Nitrate                                                                                        | <b>1.16971</b> |
| <b>Sample Preparation</b>                                                                                                              |                |
| Calcium chloride dihydrate for analysis EMSURE® ACS, Reag. Ph Eur                                                                      | <b>1.02382</b> |
| MQuant® Nitrate Test, 10–500 mg/L Nitrate                                                                                              | <b>1.10020</b> |
| Distilled water (e.g., from a suitable Milli-Q® system) or water for analysis                                                          | <b>1.16754</b> |
| <b>Analytical Quality Assurance (AQA)</b>                                                                                              |                |
| RQcheck set for RQflex® 20 Reflectometer                                                                                               | <b>1.17247</b> |
| Recalibration Set for RQflex® 20 Reflectometer                                                                                         | <b>1.16954</b> |
| Nitrate standard solution CRM 1000 mg/L (NO <sub>3</sub> ), traceable to SRM from NIST NaNO <sub>3</sub> in H <sub>2</sub> O Certipur® | <b>1.04613</b> |



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