CUSTOMBIOTECH

Roche

Nitrate Assay for Cedex® Bio & Cedex® Bio HT Analyzers

Reliable and convenient automated determination

Nitrate is an important source of accessible nitrogen in culture media of plant cells (e.g. algae cultures) and generally in plant breeding. Due to the fast consumption, the cultures require continuous feeding of the appropriate amounts of nitrate. For humans and animals, nitrate in water and food should be avoided due to its potentially harmful effects, therefore, nitrate is an important parameter in the analytics of food, drinking water and environmental water.

In culture media for eukaryotic cells or for microbial fermentation some metal cations are often added as nitrate salts because of the good solubility. However, nitrate is not beneficial for the cultures and the nitrate content should be checked in the quality control of the media.

Process control based on fast and reliable analytics

- Automated assay providing accurate results
- Wide measuring range, high sensitivity, option for automated pre-dilution
- Low sample volume of 2 – 20 µL used

Assay principle

For determination of the nitrate concentration, the nitrate from the sample is reduced to nitrite by NADPH in presence of aspecific nitrate reductase enzyme (NR). The consumption of NADPH is measured photometrically by the decrease of the absorbance at 340 nm, directly correlating to the nitrate concentration in the sample.

$$\text{Nitrate} + \text{NADPH} + H^+ \xrightarrow{\text{NR}} \text{Nitrite} + NADP^+ + H_2O$$

Nitrate test ranges

In order to generate highly accurate results over a wide nitrate concentration range, three instrument protocols are provided for a low, standard and high target range.

Protocol	Concentration range
NO3L (low range)	0.2 – 4 mmol/L, 12 – 248 mg/L
NO3B (standard)	1 – 25 mmol/L, 62 – 1,550 mg/L
NO3D (high range)	15 – 250 mmol/L, 930 – 15,503 mg/L, and up to 2.5 mol/L, 155 g/L with auto-dilution

High precision

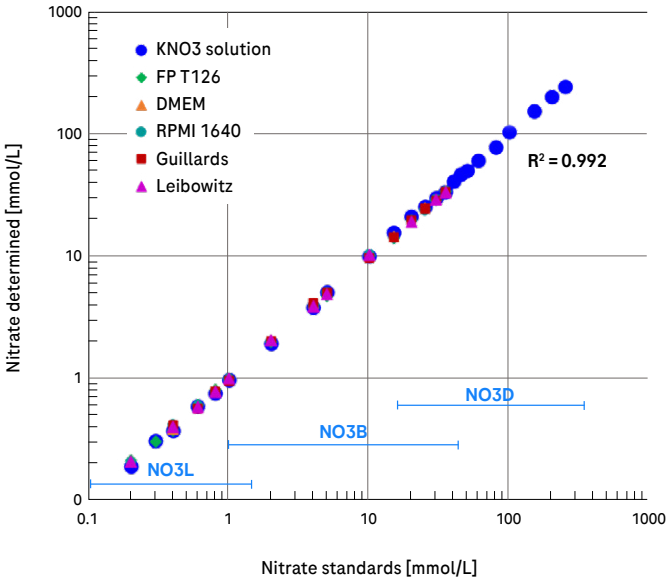
Nitrate was determined in spiked culture media samples on a Cedex Bio HT Analyzer. Coefficients of variation (CV) are calculated for 21 replicates in one run, and repeated determination on 10 days. (Roche evaluation data)

	Level 1	Level 2	Level 3
Nitrate conc. [mmol/L]	2.3	11.1	20.2
CV in-run	1.1 %	1.6 %	1.0 %
CV, inter-run	6.5 %	4.2 %	4.3 %

For use in quality control/manufacturing process only.

High accuracy

Throughout the wide test range, nitrate concentrations can be determined with high accuracy in various media matrices.

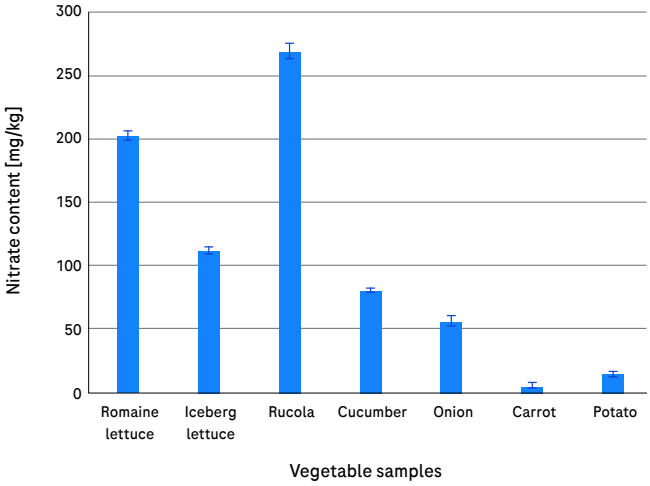


01

Recovery of nitrate: Nitrate was determined on a Cedex Bio Analyzer in rows of standards with increasing nitrate concentrations spiked into several types of culture media. The protocols NO3L, NO3B and NO3D were used for the low, medium and high range, respectively. The results show a perfect test accuracy over the whole concentration range, independently of the different media formulations. All samples were determined with deviations < 6 % from the target concentrations. (Roche evaluation data)

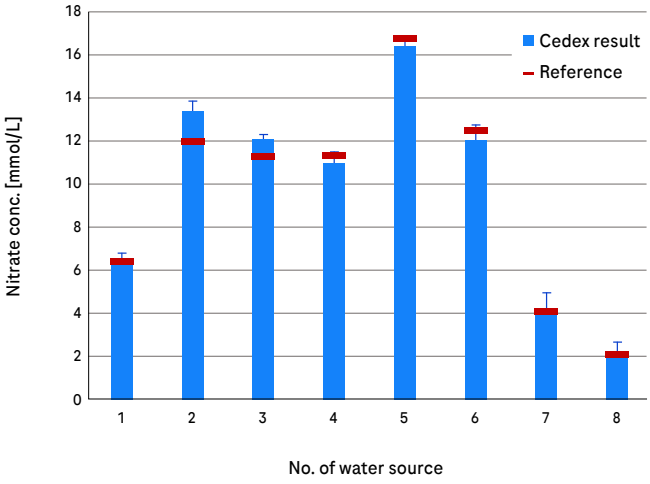
Reliable analytics for various applications

The provided test protocols enable the accurate and precise determination of nitrate over a wide concentration range, suited for nitrate monitoring in plant culturing, the analysis of food stuff extracts, control of cell culture media, and testing of tap water or environmental water specimen.



02

Nitrate in vegetables: Nitrate was determined on a Cedex Bio HT Analyzer in water extracts of ground samples of different vegetables, five replicates each. The determined concentrations were used to calculate the nitrate content of the vegetable samples. (Roche evaluation data)



03

Nitrate in tap water: Nitrate was determined on a Cedex Bio HT Analyzer in samples of tap water, five replicates each, from several locations (different cities, two with mountain spring water), compared to reference values in water quality reports of the local authorities. Even for such low concentrations, the Cedex results are highly accurate and precise. (Roche evaluation data)

Potential interferences

- **Nitrite (NO_2^-)**
  If nitrite is present in a concentration equal or higher than the nitrate concentration, then the nitrate test result may be too high. For example, a solution containing 3 mmol/L each of nitrate and nitrite resulted in 3.63 mmol/L (121 % recovery).
- **Cyanide (CN^-)**
  Cyanide concentrations ≥ 2 mmol/L cause an inhibition of the nitrate reductase and the recovery in the nitrate test will be too low.

Ordering information
For determination of nitrate the following products are required in addition to the Cedex Analyzer with the general system reagents and accessories:

Product	Pack size	Catalog Number
Nitrate Bio	4 x 50 tests	09 816 364 001
Nitrate Bio HT	300 tests	09 816 356 001
Calibrator D Bio	6 x 1 mL	07 368 321 001
Control D Level 1 Bio	6 x 1 mL	07 368 178 001
Control D Level 2 Bio	6 x 1 mL	07 368 186 001
Control D Level 3 Bio	6 x 1 mL	07 368 194 001
Chimneys (for Nitrate Bio)	96 pieces	06 455 646 001

Regulatory Disclaimer
For use in quality control/manufacturing process only.

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CEDEX is a trademark of Roche.

Scan for ordering information for all Cedex Bio Analyzer and Cedex Bio HT Analyzer assays

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