EN

TOTAL OXIDIZED NITROGEN (ENZYMATIC)

❶ AtNaR and NADH: 984187
❷ TON R3: 984371

INTENDED USE
For determination of the total oxidized nitrogen in drinking, ground, surface and waste water on Thermo Scientific™ Aquakem™ or Gallery™ analyzers.

METHOD
Colorimetric method.

PRINCIPLE OF THE PROCEDURE
Nitrate analysis is accomplished by reduction of nitrate to nitrite, and the reaction of the resulting nitrite with the Griess reagents to yield a highly coloured compound which can be quantified using a spectrophotometer. In the AtNaR (enzyme) Nitrate Analysis Method, the enzyme nitrate reductase catalyses the reduction of nitrate to nitrite with the natural reducing agent of this enzyme, NADH (reduced nicotinamide dinucleotide), to drive the conversion (Campbell et al, 2006a).

REAGENT INFORMATION
AtNaR and NADH (984187) and TON R3 (984371) need to be ordered separately.

Product 984187 contains:

AtNaR	3 U/vial
Enzyme diluent bulb	1 vial
NADH	2 mg/vial

For this method, additionally needed items:

- Eppendorf tubes, 1.5 ml, 6 pcs
- Pipette, e.g. Thermo Scientific™ Finnpipette™ F1 100-1000 µl
- Reagent glass vessel, 10 ml (5 pcs), code 984050

Ready-to-use reagents		Barcode id
984371 TON R3	4 x 20 ml	A08

Note: Check that there are no bubbles on the surface of the reagent when you insert vials in the analyzer.

Precautions
Exercise the normal precautions required for handling all laboratory reagents.
The products have to be disposed of as laboratory chemical in accordance with local regulations.

SOLUTIONS NEEDED
Disodium ethylenediaminetetraacetate dihydrate (EDTA) 100 mM
Dissolve 3.72 g EDTA (MW=372.24, Ultrapure grade) in approximately 80 ml deionized water and dilute to 100 ml with DI water. This reagent is stable at room temperature for 1 year.

Phosphate Buffer pH 7.5 (EDTA 5 mM)
Dissolve 3.75 g potassium di-hydrogen phosphate (KH_2PO_4 FW=136.1) and 1.4 g potassium hydroxide (KOH, FW=56.11) in approximately 800 ml of DI water in a 1000 ml volumetric flask. Add 50 ml of 100 mM EDTA and dilute the solution to the mark with DI water and mix well.

By adjusting the concentration of the EDTA in this solution it can assist with raising and lowering the analysis range.

This solution is stable at room temperature for 1 year.

ENZYME PREPARATION
Nitrate Reductase Stock Solution (AtNaR in foil)
Empty the contents of the Enzyme diluent bulb by squeezing the bulb into the AtNaR vial.

Mix gently by hand for 10 seconds. Allow to stand at room temperature for minimum of 10 minutes with mixing at 5 and 10 minutes.

AtNaR reconstituted with the Enzyme Diluent can be frozen and thawed repeatedly. It also stabilises AtNaR so you don't need to keep it on ice during use.

NADH Stock Solution (NADH in foil)
Add 1.0 ml of Phosphate Buffer to the NADH vial (2 mg NADH). Store unused reagent in a freezer.

REAGENT PREPARATION
Prepare working solutions from the AtNaR and NADH reconstitutes according to the table below.

	AtNaR Working solution		NADH Working solution	
No Assays	**AtNaR Enzyme**	**Phosphate Buffer**	**NADH**	**Phosphate Buffer**
330	1.0 ml vial	19.0 ml	1 ml vial	9.0 ml
100	335 µl	6.3 ml	335 µl	3.0 ml
50	168 µl	3.15 ml	168 µl	1.5 ml

Working solutions are stable on board for approximately 8 hours.

Storage and Stability
Ready-to-use reagent TON R3
Reagents in unopened vials are stable at 2…8 °C until the expiry date printed on the label.
See the Application Note for the reagent on-board stability.

Dry AtNaR (Nitrate Reductase Enzyme)
AtNaR is provided freeze dried in vials. Storage of AtNaR in a refrigerator or freezer is recommended.

Dry AtNaR stored at 20 - 25 ºC is stable for 6 months.
AtNaR stored at 4 ºC is stable for 1 year.
AtNaR stored at –20 ºC (standard freezer) is stable for 2 years.
AtNaR stored at –80 ºC or colder is stable for 3 years.

AtNaR Stock solution
AtNaR stock solution prepared using the Enzyme Diluent solution can be stored in a freezer.

Reconstituted AtNaR stored at –20 ºC (standard freezer) is stable for 6 months. AtNaR stored at –80 ºC or colder is stable for 1 year.

The enzyme diluent contains glycerol and other protein stabilisers – Never freeze the enzyme solution in buffer alone, always use the diluent.

NADH Stock solution
NADH Stock solution can be stored at -20 ºC (standard freezer) for 1 month.

SAMPLES
Sample type
Drinking, ground, surface and waste water.

Sample preparation
Sample material should be homogenous and representative.

TEST PROCEDURE
See a separate Application note for Aquakem or Gallery analyzer. Application note is suggestive and should be tailored to sample matrix and concentration in use.

Materials required but not provided
Deionized water (aseptic and free of heavy metals) and general laboratory equipment.

Standard solutions available:
984722 Nitrite (as NO_2) Std, 1000 mg/l
984723 Nitrite (as N) Std, 1000 mg/l
984724 Nitrate (as NO_3) Std, 1000 mg/l
984725 Nitrate (as N) Std, 1000 mg/l

Calibration
Calibration is polynomial/2nd order.

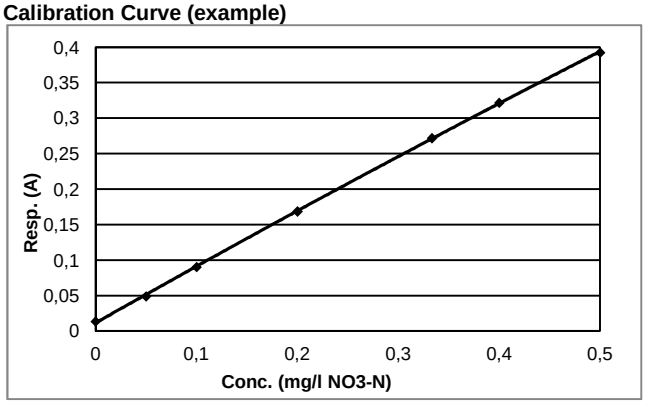
For Aquakem TON Enz Low Application, a 5000 µg/l as N calibration standard was used.
For Aquakem TON Enz High Application, a 10 mg/l as N calibration standard was used.
For Gallery Application TON Ezymatic, a 2 mg/l as N calibration standard was used.

Calibrators can be diluted automatically by the analyzer or manually by the user.

Quality Control
Use appropriate quality control samples to verify calibration and to ensure ongoing performance. Run quality control samples always after each calibration, before the daily sample load and in suitable intervals, e.g. every 10th sample, to verify the reagent on board stability. Run QC samples also every time a new reagent vial is used. It is also recommended to use two levels of controls. The control intervals and limits must be adapted to the individual laboratory requirements. The results of the quality control sample(s) should fall within the limits pre-set by the laboratory.

Calculation of results
The results are calculated automatically by the analyzer using a calibration curve.

Calibration Curve (example)



Note that the calibration curve is lot dependent. This calibration curve is performed by Gallery analyzer.

PERFORMANCE CHARACTERISTICS
The results obtained in individual laboratories may differ from the performance data given.

MEASURING RANGE

Analyzer	Range as Nitrogen (N)	Extended measuring range
Aquakem	TON Enz Low * – 500 µg/l N	Up to 5000 µg/l N
Aquakem	TON Enz High * - 30 mg/l N	Up to 10 mg/l N
Gallery	TON Enzymatic *- 0.5 mg/l N	Up to 2.5 mg/l N

Quantitation Limit
The quantitation limit is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit can be estimated for example by multiplying 5 to 10 times the SD of a blank sample.

Method Detection Limit (MDL)
The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

Application	Sample	n	Average (mg/l N)	SD	MDL (mg/l N)
TON Enzymatic	**blank**	**7**	**-0.00007**	**0.00011**	**0.00035***

MDL was determined using Gallery analyzer.
*MDL = 3.14 x SD (blank sample, n = 7)

LIMITATIONS OF THE PROCEDURE

Interference
The pH of the sample must be between 6 and 8 to avoid denaturing the enzyme.

OTHER REMARKS
Note that the application performance has been verified with pure chemicals dissolved in deionized water and with spiked native samples. The results obtained in individual laboratories may differ from the given performance data due to e.g. sample matrix, concentrations or analysis environment. Each laboratory is responsible to verify the method to prove the analysis performance.

WASTE MANAGEMENT
Please refer to local legal requirements. It is recommended to empty the analyzer cuvette waste bin and waste water daily. Emptying should be done immediately after the analysis when using hazardous reagents/solutions.
Note: If using reagents/solutions that react with each other, cuvette waste bin and waste water should be emptied and washed between use of these reagents.

BIBLIOGRAPHY
1. USGS-NWQL I-2547-11: Nitrate Plus Nitrite in Water by Enzymatic Reduction, Standard Level, Auto Analyzer
2. USGS-NWQL I-2548-11: Nitrate Plus Nitrite in Water by Enzymatic Reduction, Low Level, Auto Analyzer
3. ASTM D7781-14: Standard Test Method for Nitrite-Nitrate in Water by Nitrate Reductase
4. Method for Nitrate Reductase Nitrate-Nitrogen Analysis of Drinking Water, Version 1.0, Revision 2.0, Feb. 2016. Nitrate Elimination Company, Inc. (NECi).

ADDITIONAL MATERIAL
Certificate of analysis and SDS are available at
www.e-labeling.eu/TSF

Applications for Gallery and Arena automated analyzers are available upon request from the local sales representative. Information in the Application note can change without prior notice.

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Changes from previous version
Added instruction for Quality Control procedures.
Reagent stability updated.
Bibliography reference no 4 added.
Contact info updated.
General updates.