



NECi: The Nitrate Elimination Company, Inc.

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Autoanalyzer Reagent Kits for Discrete Analyzers (DA-ARK) Konelab Kit #800303-03P

Introduction

NECi Nitrate Reductase (NaR) Nitrate Analysis Method has been demonstrated to match cadmium reduction for nitrate determination using discrete analyzer technology (Patton et al., 2007; Campbell et al., 2006b). Enzyme-based nitrate analysis overcomes the difficulties cadmium reduction presents for discrete analyzer technology. In addition, NECi's Method is more environmentally benign. NECi's new NaR, AtNaR, makes nitrate analysis at 37°C easy and reliable.

Method

Nitrate analysis is accomplished by reduction of nitrate to nitrite, and reaction of the resulting nitrite with the Griess reagents to yield a highly colored compound which can be quantified by using a colorimeter or spectrophotometer. In the NECi NaR Nitrate Analysis Method, the enzyme nitrate reductase catalyzes 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). Since all the reagents for the NECi NaR method are water soluble and stable in solution, the method is ideally suited for discrete analyzers (Campbell et al., 2006b).

Reagents

Reagents for NECi NaR Nitrate Analysis Method for use with discrete analyzers are supplied in ready-to-use Automated Reagent Kits, called DA-ARKs. The DA-ARK1 is designed for the KoneLab instrument series.

DA-ARK1 (Catalog number 800303-03P) contains sufficient reagents for 330 analyses, and includes:

- NaR Reagent – 3 units AtNaR in dry form in vacuum pack
- Special enzyme diluent – 1 vial
- NADH Reagent – 2 mg in dry form in vacuum pack

Detailed instructions including reconstitution of the NaR and preparation of all reagents are included with the kits and summarized on the reverse of this flyer. **Discounts** are available for bulk orders. See NECi's website or call for pricing.

Instruments

The following discrete analyzers are "qualified" to run the NECi NaR Nitrate Analysis Method:

- Konelab Aquakem and Konelab Arena (see reference 3 below)
- OI Analytical Inc DA3500
- Lachat AP300 (see reference 2 below, instrument is discontinued)

Other discrete analyzers such as those sold by Astoria-Pacific, Systea, Seal Analytical, Westco Sci Instruments, and others will soon be qualified using the method – ask your sales representative for details.

References

1. Campbell, Wilbur H., GG Barbier, P Song (2006a) Nitrate Reductase for Nitrate Analysis in Water. Environmental Chemistry Letters 4:69-74.
2. Campbell, Wilbur H., Ellen R. Campbell, Lynn Egan (2006b) Green Chemistry Nitrate Determination: An Alternative Nitrate Analysis Method. American Laboratory, February, 2006.
3. Campbell, Wilbur H., ER Campbell (2007) Nitrate Analysis using Different Nitrate Reductase Isozymes. American Lab Aug07 pp45-46.
4. Patton, C. J., et al. (2008) Nitrate Analysis with Nitrate Reductase on the Discrete Analyzer. In preparation.

Reagents:

You'll need to prepare these buffers and reagents:

- **Ethylenediamine tetraacetic acid (EDTA, 25 mM):** Dissolve 9.3 g Ultrapure EDTA (FW = 372.24) in approximately 800 mL deionized water (DI water) contained in a 1 L volumetric flask. Dilute to the mark with DI water and mix well. Transfer to a bottle and store at room temperature. Stable for one year.
- **Phosphate Buffer (pH = 7.5):** Dissolve 3.75 g potassium di-hydrogen phosphate (KH₂PO₄, FW = 136.1) and 1.4 g potassium hydroxide (KOH, FW = 56.11) in about 800 mL of DI water contained in a 1 L volumetric flask. Add 1 mL of the 25 mM EDTA and dilute the resulting solution to the mark with DI water and mix it well. Transfer this solution to a bottle where it is stable at room temperature for about 1 year.
- **Sulfanilamide (SAN):** Pour 300 mL of concentrated HCl into about 500 mL of deionized water contained in a 1 L volumetric flask. Swirl the flask to mix its contents. Dissolve 10 g SAN (FW = 172.2) in the resulting HCl solution, dilute it to the mark with DI water, and mix it well. Transfer this solution to a bottle where it is stable at room temperature for at least six months.
- **N-(1-Naphthyl)ethylenediamine dihydrochloride (NED):** Dissolve 1 g of NED (FW = 259.2) to about 800 mL of deionized water contained in a 1 L volumetric flask. Dilute the resulting solution to the mark with DI water and mix it well. Transfer this solution to a brown glass bottle where it is stable at room temperature for at least six months.

Protocol for the KoneLab Aquakem using NECi's DA-ARK #800303-03P:

The Reagent Kit contains NADH and AtNaR in vacuum-sealed pouch, enzyme diluent, and instructions.

1. Reconstitute AtNaR enzyme [clear vial, red cap] using Enzyme Diluent supplied with the kit (detailed instructions included in pack). This gives you a Stock Solution of 3.0 Units/ml. Store this solution in a freezer between uses. Enzyme diluent contains glycerol and other protein stabilizers. **Never freeze enzyme solution in buffer alone! Use the diluent!**
2. To make sufficient reagents for 8 hours of run time, or ~330 assays, transfer contents of the AtNaR Stock Solution to 19 ml Phosphate Buffer (as prepared above) for a total volume = 20 ml.
 - a. For fewer samples or shorter run times, use smaller volumes (e.g. 335 µl AtNaR Stock Solution and 6.3 ml Phosphate buffer for 100 assays in 2.5 hr).
3. Add 1.0 ml Phosphate Buffer to NADH vial [2.0 mg NADH in amber vial, blue cap]. For 8 hr/330 assays, dilute to 10 ml total volume using Phosphate Buffer (above)
 - a. For 100 assays/2.5 hr, add 335 µl of this NADH solution to 3.0 ml buffer. Store unused reagent in a freezer.

Reagent prep for making your own NADH and NaR Solutions:

- **NADH Stock (Sigma #N-8129):** Dissolve 0.100 g of NADH (FW = 709.4) in approximately 40 mL of DI water contained in a 50-mL volumetric flask (final concentration 2 mg NADH/ml). Dilute to the mark and mix well. Transfer 1-mL aliquots to 1.5-mL snap-top vials and store at -20°C. Stocks stable for 1 month.
 - a. Working solution: 1 (1 mL) aliquot/9 mL phosphate buffer (10.0 mL total volume).
- **Nitrate reductase from *Arabidopsis thaliana* (AtNaR, NECi #800303):** Add 1 mL of phosphate buffer to a 3.0-unit vial of AtNaR. Gently shake the vial several times over the course of at least 10 minutes to affect dissolution. Add the reconstituted enzyme to 19 mL phosphate buffer (20 mL total volume). Rinse the vial several times with aliquots of the final enzyme/phosphate buffer solution. After freeze-dried NaR is reconstituted in phosphate buffer, it is stable for about 8 hours at 4°C. Sufficient for about 330 analyses.

Reaction Conditions for the KoneLab AquaKem

1. 55 µL AtNaR Solution
2. 5.0 µL Sample. Mix.
3. 15 µL NADH. Mix.
4. Incubate 600 s at 37°C
5. Add 25 µL SAN. Mix and incubate 120 s at 37°C.
6. Add 25 µL NED. Mix and incubate 120 s at 37°C.
7. Measure absorbance at 540 nm, using secondary wavelength correction at 700 nm.

For other instruments, modify volumes as required using above as a base.

Call NECi for info about adapting method to other DA instruments!

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