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Instruments

Compatible with Seal Discrete Analyzers

SKU: NRPk-SA-1At

Introduction

Nitrate Reductase Reagent Packs for Discrete Analyzers were developed by NECi in conjunction with the USGS to replace toxic cadmium in automated colorimetric nitrate determination methods. All reagents for the nitrate reductase method are water soluble and stable in solution, making this method optimal for use in discrete analyzers (Campbell et al., 2006b). NECi's Nitrate Reductase from Arabidopsis thaliana (AtNaR) is a recombinant enzyme produced by strict QA/QC procedures with reagent grade water, sugars, and salts to ensure lot-to-lot consistency, reliability, and stability. Nitrate Reductase is non-toxic and environmentally benign.

Method

Nitrate Reductase catalyzes the reduction of nitrate to nitrite with the natural electron donor, NADH (reduced nicotinamide dinucleotide) to drive the conversion (Campbell et al., 2006a). Nitrite is then reacted with sulfanilamide to form a diazo compound which is then reacted with N-(1-napthyl)-ethylenediamine dichloride to produce a pink color. This color is then quantitatively measured using a colorimeter or spectrophotometer reading absorbance at 540 nm ±20 nm. Results are then analyzed by generating a standard curve using a spreadsheet program or statistics package.

Reagents Included

This Reagent Kit contains **Nitrate reductase from *Arabidopsis thaliana* (AtNaR)**, enzyme diluent, NADH, and instructions:

- AtNaR – 1 unit/vial (freeze-dried) in vacuum sealed foil pouch
- Enzyme diluent – 1 squeeze bulb (pre-measured) with reconstitution instructions
- NADH – 4.8 mg (dry powder in vial) in vacuum sealed foil pouch

Preparation of Additional Reagents are explained in detail on the reverse of this sheet

Instrument

This kit (*NRPk-SA-1At*) is designed for ~85 assays with Seal discrete analyzers. Reagent Packs with detailed instructions are also available for other discrete analyzers, flow injection analyzers, continuous and segmented flow analyzers, and other automated laboratory instruments.

Please call NECi about adapting our method for other automated laboratory instruments or ask your sales representative.

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.

Preparation of Buffers and Reagents (not provided in pack):

- **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 (KH_2PO_4, 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 0.2 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.

Preparation of Enzyme Reagents (AtNaR and NADH):

1. Reconstitute AtNaR enzyme [clear vial, green cap] using Enzyme Diluent supplied with the kit (detailed instructions included in pack). This gives you a Stock Solution of 1.0 Unit/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 ~85 assays, transfer contents of the AtNaR Stock Solution to 11 ml Phosphate Buffer (as prepared above) for a total volume of 12 ml.
    a. For fewer samples or shorter run times, use smaller volumes (e.g. 500 µl AtNaR Stock Solution and 5.5 ml Phosphate buffer for ~42 assays).
3. Add 1.5 ml Phosphate Buffer to NADH vial and mix gently by hand. Then, transfer this solution to a larger tube and dilute to 12 ml total volume in Phosphate Buffer to make a final concentration of 0.4 mg NADH/ml. Store unused reagent in a freezer.

Protocol for preparing your own NADH and Nitrate Reductase Reagents:

- **NADH Stock (Sigma #N-8129):** Dissolve 0.024 g of NADH (FW = 709.4) in 5 mL of Phosphate Buffer for a concentration of 4.8 mg NADH/ml). Transfer 1.0 mL aliquots to 2.0 mL snap-top vials and store at -20°C. Stocks stable for 1 month. Thaw and dilute to 12 mL with assay buffer as needed.

- **Nitrate reductase from *Arabidopsis thaliana* (AtNaR)**: Add 1 mL of phosphate buffer to a 1.0-unit vial of AtNaR. Gently invert the vial several times over the course of at least 10 minutes to affect dissolution. Add the reconstituted enzyme to 11 mL phosphate buffer (12 mL total volume). After freeze-dried AtNaR is reconstituted in phosphate buffer, it is stable for about 8 hours at 4°C. This solution is sufficient for ~85 analyses.

Reaction Conditions (prior to a run, combine an appropriate volume of SAN and NED 1:1 for use below)

1. 175 µL Phosphate Buffer.
2. 25 µL Sample. Mix.
3. 30 µL NADH. Mix.
4. 140 µL AtNaR. Mix.
5. Incubate 600 seconds.
6. Add 135 µL of mixed SAN/NED and mix.
7. Incubate 120 seconds.
8. Measure absorbance at 540 nm, using secondary wavelength correction at 700 nm.

To adapt this assay to other instruments, modify volumes as required, keeping the above ratios equivalent. Contact NECi directly for assistance modifying this method to other automated laboratory instruments or to build a custom reagent pack at no extra cost.