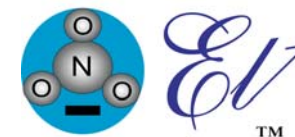


# Nitrate Analysis in Difficult Matrices Using Enzymatic Reduction Method

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## The Nitrate Problem



## Nitrate Analysis Methods

Nitrate analysis is one of the most commonly done methods in environmental analysis. Cadmium is widely used in nitrate analysis. Cadmium is a toxic pollutant. An alternative method is the Enzymatic Reduction Method using Nitrate Reductase (NaR). NECi first produced recombinant YNaR1 in the host *Pichia pastoris* (1). This Superior Stock NaR was a major improvement over natural corn leaf NaR (2). Recently, NECi started production of a new recombinant NaR, which is called AtNaR2. This new Superior Stock NaR has robust activity at 37°C (3).

The two Superior Stock NaRs complement each other: YNaR1 is most effective for nitrate analysis at room temperature; AtNaR2 is most effective at higher temperatures and in difficult matrices like saltwater and other salt-containing aqueous extracts.

## Enzymatic Reduction Method for Nitrate Analysis

Nitrate Reductase (NaR) catalyzes reduction of nitrate to nitrite with NADH as cofactor. NaR and NADH replace toxic Cadmium. The resulting nitrite reacts with Griess reagents to produce a highly colored product. Thus, there is little change from the Standard method, except there are no toxic reagents!

### References:

1. BARBIER, G. G., and CAMPBELL, W.H., "Expression of *Pichia angusta* nitrate reductase (YNaR1) in *Pichia pastoris*." Current Topics in Gene Expression Systems: Program and Abstracts, Abstract PY 4, p. 64 (2000).
2. CAMPBELL, E. R., T. KINNUNEN-SKIDMORE, WINOWIECKI, L. A., and CAMPBELL, W. H., A New Trend in Nitrate Analysis: An Enzyme-based Field Test for Nitrate. American Laboratory (News Edition) 33 (4): 90-92 (2001).
3. Campbell, Wilbur H., GG Barber, P Song (2006) Nitrate Reductase for Nitrate Analysis in Water. Environmental Chemistry Letters, 4: 69-73.

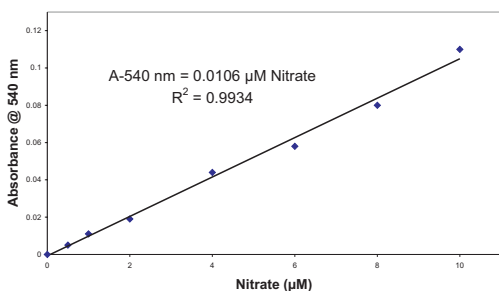
## Recent NECi Innovations

Production of a new NaR - AtNaR2  
Nitrate Analysis Reagents for Discrete Analyzers  
NaR Nitrate Analysis at 37°C.  
Nitrate Analysis in Saltwater and Soil Extracts

# NECi manufactures Superior Stock Nitrate Reductase for Nitrate Analysis and Nitrate Test Kits

## I. Nitrate Analysis in Seawater.

Ultra-Low Nitrate in Seawater



Nitrate analysis standard curve for seawater. Nitrate standards were made up in Instant Ocean. Superior Stock Nitrate Reductase (NaR) was used at 0.02 units per assay (ie. double the std level).

## II. Nitrate Analysis in Soil Extracts.

### Nitrate Content of Commercial and Test Soils

Soil Sample Extracted	Mean Nitrate Content (ppm N)	Std Error (N = 5)	Soil N Credit (lbs N per acre)
Miracle Gro Potting Mix	50.7	1.2	304
Premium Planters Mix	33.0	0.9	198
High N Soil	82.8	2.3	497
Low N Soil	1.02	0.03	6

Soils were extracted with deionized water and filtered (1 g soil per 5 mL water).

Nitrate was analyzed using NECi Ag-S-NTK. Soil extracts were diluted 5-fold for analysis.

## NaR vs Cadmium for Nitrate Analysis

Characteristic	NaR	Cd
Sustainable	Yes	No
Efficiency	Catalytic	Stoichiometric
Reaction type	Homogeneous	Heterogeneous
Specificity	High	High (?)
Reproducibility	High	Variable
Toxicity	None	High
Waste	Biodegradable	Persistent
Interferences	Few	Many
Sample size	μL	mL

**NaR-based Nitrate Analysis Methods are the Greener Choice!**