

Replace Cadmium with Enzymes for Nitrate Analysis

- ✓ Minimizes Hazardous Waste & Handling
- ✓ Increases Lab Safety
- ✓ Provides Accurate & Reliable Data

Nitrate reductase is an enzyme that accurately quantifies nitrate in many sample types. NECi Superior Enzymes' enzymatic nitrate quantification method has been validated for environmental testing, industrial quality, academic research and more in various on-site and laboratory formats.

- ▶ **Nitrate reductase catalyzes the reduction of nitrate to nitrite** in the presence of NADH
- ▶ **This is a thermodynamically irreversible reaction**
- ▶ Sequential addition of Griess reagents **produces a chromophore**
- ▶ **This pink chromophore is measured spectrophotometrically at 540 ± 20 nm.**
- ▶ Calibrants of certified nitrate standard are used to yield a standard curve
- ▶ Samples are compared to express sample concentrations as mg/L nitrate-N.



WHY ENZYMATIC NITRATE ANALYSIS?

-  **HIGH SENSITIVITY**
Low detection limits even in complex sample matrices, nitrate reductase completely reduces all nitrate in sample to nitrite
-  **STABILITY IN STORAGE & SOLUTION**
Nitrate reductase is supplied as highly purified and stable freeze-dried protein glass, also offering stability in solution
-  **LESS INTERFERENCES**
Chloride, fluorine, bromine, sulfate, iron, zinc, aluminum, bromate, chlorine dioxide, and chlorate all showed no interference with the enzymatic method
-  **NATURAL SPECIFICITY**
Enzymes have a specific active site which only binds to the target analyte. Nitrate reductase only binds to nitrate in solution.
-  **MINIMAL SAMPLE PREP**
Most aqueous samples are ready to analyze, and plant, soil & food samples are ready for analysis after a simple water extraction
-  **NON-TOXIC & ECOLOGICALLY BENIGN**
Nitrate reductase is a naturally occurring enzyme, safe to handle, offers low reaction volumes and generates no hazardous waste
-  **HIGHLY SELECTIVE**
Enzymes are able to target analyte in complex mixtures, offering high selectivity
-  **REPRODUCIBLE RELIABILITY**
Recombinant enzymes are guaranteed consistent lot-to-lot, and offer precision in replicate sample results

METHOD VALIDATIONS



Approved Method NECi 2016a in 40 CFR Part 141 for SDWA Reporting

Accepted Method Case No. N07-0003 pending approval in 40 CFR Part 136 for CWA Reporting



Standard Test Method for Nitrate-Nitrite in Water by Nitrate Reductase



Standard + Low Level Colorimetric Determination of Nitrate + Nitrite by Enzymatic Reduction, Automated Discrete Analyzer Method

“Enzymatic Reduction Method for Nitrate-N Analysis has been validated by a robust Inter-Laboratory Study of Wastewater and Seawater Sample Matrices.” - EPA Validation Study for CWA Reporting

EPA Validation Study for CWA Reporting

SAMPLE MATRICES DETERMINED FOR PRECISION & ACCURACY

- ▶ Denver Area Treatment Plant Influent Wastewater
- ▶ Denver Area Treatment Plant Wastewater Effluent
- ▶ Michigan Paper Mill Waste Stream Effluent
- ▶ Denver Area Metal Finisher Waste Stream Effluent
- ▶ Denver Area Commercial Laundry Waste Stream Effluent
- ▶ Environmental Resources Associates #507 Hardness Wastewater Reference Material
- ▶ Michigan Confined Animal Feeding Operation (CAFO) Effluent from Tiled Field
- ▶ Low-Nutrient Seawater (Offshore Hawaii)
- ▶ ERA #608 Reference Standard
- ▶ USGS PE N-116 (Low Nutrient-Fortified River Water)
- ▶ USGS PE N-115 (High Nutrient-Fortified River Water)
- ▶ Various Municipal Drinking Water Samples
- ▶ Various Geological & Environmental Water Samples
- ▶ Aqueous Extracts of Food, Animal Forage, Milk, and Soil Samples
- ▶ Various Industrial Processing Waste Effluent Samples

Learn more at www.nitrate.com/nitrate-test-method-validations