NECi is an environmental biotechnology company.

We are dedicated to the application of biotechnology to the solution of environmental problems.
Why use Enzymes?

• **Selectivity**
  “Find” target in complex mixtures

• **Sensitivity**
  Low detection limits in complex mixtures

• **Specificity**
  An enzyme will react ONLY with its target

• **Safety**
  Accurate, reliable, & environmentally benign
NECi’s focus is on nitrate.

We are moving enzyme-based nitrate analysis from the R&D and clinical lab out into the wider analytical chemistry community.
NECi’s focus is on nitrate analysis using recombinant Nitrate Reductase S-NaR1b 3-D Structure

Fischer et al., 2004
3-D Model of Holo-NR Dimer
NECi Production of Nitrate Reductase by Fermentation
## Metal vs NaR for Nitrate Analysis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>NaR</th>
<th>Cd, Zn, etc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficiency</td>
<td>Catalytic</td>
<td>Stoichiometric</td>
</tr>
<tr>
<td>Reaction Type</td>
<td>Homogeneous</td>
<td>Heterogeneous</td>
</tr>
<tr>
<td>Specificity</td>
<td>High</td>
<td>Good</td>
</tr>
<tr>
<td>Reproducibility</td>
<td>High</td>
<td>Variable</td>
</tr>
<tr>
<td>Toxicity</td>
<td>None</td>
<td>High</td>
</tr>
<tr>
<td>Interferences</td>
<td>Few</td>
<td>Many</td>
</tr>
<tr>
<td>Sample Size</td>
<td>Microliters</td>
<td>Milliliters</td>
</tr>
<tr>
<td>Sustainable</td>
<td>YES</td>
<td>NO</td>
</tr>
</tbody>
</table>
Absorbance @ 540 nm

Nitrate Content (ppm nitrate-N)

Absorbance @ 540 nm

Nitrate Content (ppm nitrate-N)
Nitrate by CFA: Correlation between split samples

Nitrate + Nitrite Concentration in mg-N/L (CdR)

Nitrate Concentration in mg-N/L (NaR)

Line of equal correlation
Recombinant Nitrate Reductase

- Increased production capacity
- Guaranteed lot-to-lot reproducibility
- Reasonable production costs
- Engineered for improved stability
  - Allows storage at controlled RT
  - Stable during analytical runs
  - Shipping at ambient
How the Enzyme-based Nitrate Assay Works

- Nitrate reductase (NaR) catalyzes the reduction of nitrate to nitrite. The B vitamin NADH is cofactor.
- NaR and NADH replace the toxic cadmium reagents used in conventional nitrate analysis methods.
- The resulting nitrite reacts with the Griess color reagents to produce a highly colored product.
- Minimal change required from standard methods.
Ultra Low Range Nitrate Assay with NADH/NaR

Absorbance @ 540 nm

µM Nitrate

0 2 4 6 8 10

Absorbance @ 540 nm

0.00 0.02 0.04 0.06 0.08 0.10

0 2 4 6 8 10

µM Nitrate
Why Nitrate?

- US EPA Primary water contaminant
- Toxic to infants at 10 ppm in water
- High levels toxic to livestock
- Produced in waste streams of many manufacturing and ag processes
- Excess nitrate harms the environment – algal blooms, coral death, acid rain
How is nitrate measured?

- High cost eqmt: Ion Chromatography, FIA & CFA, Discrete Analyzers
- Test kits based on cadmium, hydrazine, chromotropic acid, brucine, or zinc
- Ion selective electrode
- Test strips
- And nitrate reductase, which can be used in many of these methods
Environmental + Water Quality

Agriculture + Farmers

Nitrate Analysis Applications

Home Owners

Education + Citizen Monitoring

Utilities + Industrial Labs

Researchers + Clinical
NECi and collaborators have adapted the assay to a variety of formats and levels of skill and accuracy:

- Lab kits for quantitative data in test tube and microplate formats
- Field kits for on-site and classroom applications: provide semi-quantitative data without equipment or hazardous reagents
- Complete line of test kits for agricultural samples
- Reagent packs for automated analysis: Discrete Analyzers and Segmented Flow Analyzers
NECi has nitrate test kits for all kinds of applications
Field Kits for on-site and classroom use
Green Design

- Nonhazardous reagents facilitate use and shipping
- Minimal packaging for reduced waste and shipping costs
- Low reagent and sample volumes
- Pre-packed reagents in Field Kits improve accuracy for inexperienced users
- Specificity enables reliable results in untreated samples
Nitrate Test Kits for Agriculture

• Agricultural runoff is an important NPS of excess nutrients in the environment.
• New regulatory pressures are increasing the need for improved nutrient management on the farm.
• Nutrient costs are increasing.
• Ag producers need better tools to enable better control over nutrients.
Portable photometer for small volumes
Student Interns working on development of a nitrate test kit for on-site testing of feed crops

Many thanks to NECi collaborating labs: Dr Charles Patton, USGS NWQA Lab Dr Charles MacKown, USDA Grazinglands ARS

We thank the SBIR programs of the US Dept of Agriculture and the National Institutes of Health for their support of NECi’s R&D.
Nitrate Reductase: partial structure