



The Nitrate Elimination Co., Inc.

334 Hecla St., Lake Linden MI 49945

Tel: 906/296-1000 Fax: 906/296-8003

ellenr@nitrate.com Web: www.nitrate.com

How the Nitrate Assay Works

Enzymes are highly effective protein catalysts that accelerate very specific chemical reactions in living systems. Enzymes are biodegradable natural products, so there is little threat to the environment when enzymes and their natural substrates (reactants of the reaction catalyzed by the enzyme) are utilized for environmental testing. The enzyme used in our test kits is the enzyme nitrate reductase (NaR), which we purify from corn seedlings. In the plant, NaR is the first step toward the synthesis of the proteins and nucleic acids (DNA and RNA) the plant needs to survive.

NaR is comprised of over 900 amino acids and three co-factors: flavin adenine dinucleotide (FAD, or vitamin B2); heme iron similar to that in hemoglobin but designed for electron transfer rather than oxygen binding; and a molybdenum-containing group called molybdopterin that is found in a number of enzymes. Nitrate is reduced at molybdenum in the active site after the enzyme has been reduced by NADH (nicotinamide adenine dinucleotide, another B vitamin and a biological electron carrier). The catalytic rate of NaR is about 200 nitrate to nitrite conversions per second per molecule of NaR. The reaction is irreversible and goes to completion:



NADH is the biological electron donor. The Assay Buffer keeps the enzyme in a state near to that in the plant, so that it can react efficiently. (This step is catalyzed by a heavy metal, usually cadmium or zinc, in many conventional nitrate test kits and methods.) The resulting nitrite is then detected using standard Griess reaction chemistry. In NECi's lab format nitrate test kits, test results are read using a colorimeter or spectrophotometer. For high throughput applications, or when only very small sample volumes are available (as in biomedical research), the microplate reader can be used. Field and Consumer kits use a color chart and nitrate standards.

The Griess reaction was developed by Dr Johan Griess in the 1850's, more than 100 years ago. Nitrite in an acid solution will react with sulfanilamide and N-Naphthylethylenediamine (often called NED) to form a pink product. The intensity of the color is directly proportional to the quantity of nitrite in the solution. (The more color, the more nitrite is present.) Because we have converted (reduced) all the nitrate in the sample to nitrite using the nitrate reductase enzyme, the pink color is also directly proportional to the amount of nitrate that was in the sample. The pink color can be read by eye versus a color chart or nitrate standards. It can also be determined using a colorimeter or spectrophotometer at a wavelength of 540 nanometers.

Questions? Comments?

Visit our website at www.nitrate.com.

Or call our techline: 1.906.296.1130