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Reagent Kits for Discrete Analyzers Using NADH Disappearance

Compatible with Hitachi 717 Discrete Analyzer and other DA's measuring N by NADH disappearance

SKU: NRPk-AND-1At (Previously DA-NPk-901)

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:

Reagents for NECi NaR Nitrate Analysis Method for use with discrete analyzers are supplied in ready-to-use Automated Reagent Kits, called DA-ARKs.

This DA-ARK contains sufficient reagents for 75 analyses, and includes:

- NaR Reagent – 1 unit AtNaR in dry form in vacuum pack
- Special enzyme diluent – 1 squeeze-bulb
- NADH Reagent – 2.4 mg in dry form in vacuum pack

Detailed instructions including reconstitution of the NaR and preparation of all reagents are included with the kits and summarized on the reverse of this flyer

Reagents supplied by user:

You'll need to prepare these buffers

- **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.

Reagent preparation for the Hitachi 717 Discrete Analyzer using NECi's DA-ARK:

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. Add 1.5 ml Phosphate Buffer to NADH vial [2.4 mg NADH] and mix by inversion. Dilute to 28.5 mL total volume using phosphate buffer.

Reaction Conditions:

STEP 1 Pipette **20 µl d-I water** into one cuvette for use as reagent blank.

STEP 2 Pipette **20 µl** of your prepared **samples** and **standards** into the required number of cuvettes.

STEP 3 Add **0.38 ml diluted NADH solution** to each cuvette. Mix.

STEP 4 Zero the spectrophotometer with d-I water in a cuvette. Read absorbance of each cuvette at 340nm (A1).

STEP 5 To start the reaction add 1**0 µl NaR** solution to each cuvette. Recap and mix thoroughly.

STEP 6 Read A340 of each cuvette at exactly 20 minutes (A2) after adding NaR.

STEP 7 Read A340 of each cuvette at exactly 30 minutes (A3).

Calculations:

STEP 1 Determine absorbance differences (A1-A2) and (A2-A3) for the blank and samples.
Absorbance difference of the blank = $(A1\text{-}A2)_{blank} - 2 \times (A2\text{-}A3)_{blank}$
Absorbance difference of the sample = $(A1\text{-}A2)_{sample} - 2 \times (A2\text{-}A3)_{sample}$
$\Delta A_{nitrate}$= absorbance difference $_{sample}$ – absorbance difference $_{blank}$

STEP 2 Generate a standard curve for the Nitrate Standard (see example below). Using linear graph paper or a computer plotting program such as Sigma Plot® or spreadsheet such as Excel®, plot the ppm Nitrate-N on the x-axis, and the ΔA-340 nm for each nitrate standard on the y-axis. If plotting by hand, draw a straight line through the points for the Nitrate Standards. If plotting by computer, the slope of the line can be calculated for determining Nitrate-N ppm in the unknown samples.

STEP 3 Using the standard curve, determine the ppm Nitrate-N for the sample: (a) Find the ΔA-340 nm for the sample on the y-axis of the standard curve. (b) Follow over along a horizontal line to where the line intersects the standard curve. Trace down the x-axis and read the ppm of Nitrate-N on the x-axis.