



NECi: The Nitrate Elimination Company, Inc.

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Instructions for Discrete Analyzer Reagent Kit (D-ARK1)

D-ARK1 contains sufficient reagents for 330 analyses, and includes:

- NaR Reagent – 3 units AtNaR2 in dry form in vacuum pack
- NADH Reagent – 2 mg in dry form in vacuum pack
- Instructions for reconstitution of the NaR and preparation of all reagents

Reagents to Prepare:

- **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 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.
- **Sulfanilamide (SAN):** Pour 300 mL of concentrated HCl into about 500 mL of deionized water contained in a 1 L volumetric flask. Swirl the flask to mix its contents. Dissolve 10 g SAN (FW = 172.2) in the resulting HCl solution, dilute it to the mark with DI water, and mix it well. Transfer this solution to a bottle where it is stable at room temperature for at least six months.
- **N-(1-Naphthyl)ethylenediamine dihydrochloride (NED):** Dissolve 1 g of NED (FW = 259.2) to about 800 mL of deionized water contained in a 1 L volumetric flask. Dilute the resulting solution to the mark with DI water and mix it well. Transfer this solution to a brown glass bottle where it is stable at room temperature for at least six months.

Protocol for the KoneLab 60 using D-ARK1

1. Reconstitute NaR enzyme by adding 1 ml Phosphate Buffer Reagent and mixing vigorously 3 times over 30 min.
2. Quantitatively transfer the reconstituted NaR to 19 mL Phosphate Buffer Reagent; total volume = 20 ml. Transfer to KoneLab Reagent bottle and install in the instrument.
3. Add 1.0 ml Phosphate Buffer Reagent to NADH vial and mix completely to obtain solution, then dilute to 10 ml with Phosphate Buffer Reagent. Transfer to KoneLab Reagent bottle and install in the instrument.
4. Set up KoneLab to run NaR Nitrate Analysis Method using program provided by manufacturer.

Reaction Conditions:

1. 55 μL AtNaR2 Solution
2. 5.0 μL Sample. Mix.
3. 15 μL NADH. Mix.
4. Incubate 600 s at 37°C
5. Add 25 μL SAN. Incubate 120 s at 37°C.
6. Add 25 μL NED. Incubate 120 s at 37°C.
7. Measure absorbance at 540 nm, using secondary wavelength correction at 700 nm.

Call NECi if you have any questions!
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