

Liquid Sampling and the Sievers* Purge System

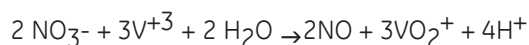
Liquid Applications

Several techniques have been developed for the measurement of NO and its oxidation products using the Sievers* Purge System in conjunction with the Sievers Nitric Oxide Analyzer (NOA). These techniques include:

- Reduction of nitrate using vanadium (III) and hydrochloric acid at 90° C
- Reduction of nitrite using iodide and acetic acid
- Reduction of nitrosothiols using a Cu(I), or Cu/Cysteine reagent, or I₃⁻/acid

Nitrates

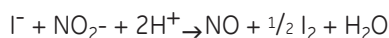
Nitrate (NO₃⁻), the major oxidation product of NO in some cell culture systems and most physiological fluids, is formed when nitric oxide reacts with oxyhemoglobin or the superoxide anion. To measure nitrate, a saturated solution of vanadium (III) chloride in hydrochloric acid converts nitrate to nitric oxide. Vanadium(III)/HCl also converts nitrite and S-nitroso compounds to NO.



To achieve high conversion efficiency, the reduction is performed at 90° C. For most applications, ~5 mL of the reducing agent is prepared in the purge vessel. That volume is usually sufficient for measurement of 20-50 samples, depending on the volume of sample injected. To prevent damage to the NOA from the hydrochloric acid vapor, a gas bubbler filled with aqueous sodium hydroxide is installed between the purge vessel and the NOA.

Nitrites

Nitrite (NO₂⁻), the major oxidation product of NO in the absence of oxyhemoglobin or superoxide anion, is formed when nitric oxide reacts with dissolved oxygen. To measure nitrite in cell culture systems, perfusates and other liquid samples, the purge vessel contains the reducing agent potassium iodide in acetic acid to convert nitrite to nitric oxide.



For most applications, ~5 mL of the reducing agent is prepared in the purge vessel. That volume is usually sufficient for measurement of 20-50 samples, depending on the volume of sample injected. The reaction is performed at room temperature.

Nitrosothiols

Nitrosothiols are important reaction products of NO and can also be measured directly using the NOA 270B, 280 or 280i and purge vessel. For lower molecular compounds like S-nitrosoglutathione, the Cu(I)/Cysteine reaction is recommended. This reduction is performed using a saturated solution of CuCl in PBS with 1mM cysteine. The technique is selective for RSNO. Standards are prepared by the reaction of thiol with nitrite. High molecular weight species like S-nitrosoalbumin or S-nitrosohemoglobin can be measured using I₃⁻/Acetic Acid reagent. Nitrite must first be removed with the Griess reagent or by column chromatography.

Liquid Accessories

Purge Vessel: The Purge Vessel, used in conjunction with the NOA Model 280i and chemical reducing agents enables the rapid reduction of all reaction products of nitric oxide. The purge vessel design ensures quantitative conversion of nitrate/nitrite/nitrosothiols to nitric oxide, and produces sharp, well-defined data peaks. Clean up is quick and simple with the built-in drain port.

Sievers Liquid Supply Kit: This kit contains the accessories necessary to perform liquid analysis. It includes one ring stand, two ring clamps, one 10 mL volumetric flask, filter paper, one 0-100 µL Eppendorf pipetter and tips, one 100-1000 µL pipetter and tips, two 10 µL Hamilton syringes, one 100 µL Hamilton syringe, 1.5 mL microtubes, and a tube rack.

Sievers Nitrate Chemical Kit: This kit contains the chemicals necessary to perform nitrate reduction with the Purge Vessel: vanadium chloride, hydrochloric acid, sodium hydroxide, and sodium nitrate.



Sievers Nitrite Chemical Kit: This kit contains the chemicals necessary to perform nitrite reduction with the purge vessel: sodium iodide, glacial acetic acid, and sodium nitrite.

NOAnalysis Software-Liquid Program

Sievers NOAnalysis Liquid Software includes custom software for liquid sampling. The Liquid program is used for all three liquid applications: nitrate reduction, nitrite reduction, and nitroso-thiol reduction.

Liquid program features include:

- Real time graphical display of NO signal
- Adjustable graphical view: zooming, panning, and x/y autoscale
- Flexible peak integration: user adjustable peak start, stop, threshold, and peak width settings
- Automatic preparation of calibration curves
- Automatic calculation of sample concentrations
- Date storage in tab-delimited text files enabling direct import to spreadsheet or statistical programs.

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USA
GE Analytical Instruments
6060 Spine Road
Boulder, CO 80301 USA
T +1 800 255 6964
T +1 303 444 2009
F +1 303 444 9543
www.geinstruments.com

Europe
Unit 3 Mercury Way
Mercury Park Estate
Trafford Park
Manchester, UK M41 7LY
T +44 (0) 161 866 9337
F +44 (0) 161 866 9630 Manchester, U.K.

MD Scientific is authorized distributor
in Denmark and Sweden for Sievers NOA 280i
www.md-scientific.dk - marekd@md-scientific.dk