Promega

Technical Bulletin

Griess Reagent System

INSTRUCTIONS FOR USE OF PRODUCT G2930.

www.promega.com

PRINTED IN USA.
Revised 6/09

Part# TB229

Griess Reagent System

**All technical literature is available on the Internet at: www.promega.com/tbs
Please visit the web site to verify that you are using the most current version of this Technical Bulletin. Please contact Promega Technical Services if you have questions on use of this system. E-mail: techserv@promega.com.**

1. Description

Nitric oxide (NO) is an important physiological messenger and effector molecule in many biological systems, including immunological, neuronal and cardiovascular tissues (1,2). Due to its involvement in these diverse systems, interest in measuring NO in biological tissues and fluids remains strong.

One means to investigate nitric oxide formation is to measure nitrite (NO_2^-), which is one of two primary, stable and nonvolatile breakdown products of NO. This assay relies on a diazotization reaction that was originally described by Griess in 1879 (3). Through the years, many modifications to the original reaction have been described.

The Griess Reagent System is based on the chemical reaction shown in Figure 1, which uses sulfanilamide and *N*-1-napthylethylenediamine dihydrochloride (NED) under acidic (phosphoric acid) conditions. This system detects NO_2^- in a variety of biological and experimental liquid matrices such as plasma, serum, urine and tissue culture medium. The nitrite sensitivity depends on the matrix (Figure 2). The limit of detection is 2.5µM (125pmol) nitrite (in ultrapure, deionized distilled water) using the protocol described in Section 4.

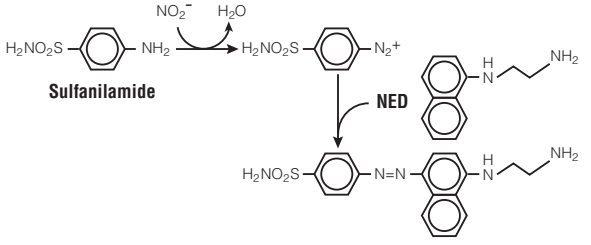

Figure 1. Chemical reactions involved in the measurement of NO_2^- using the Griess Reagent System.

Citations Using the Greiss Reagent System

- Chaea, S.Y. *et al*. (2004) Protection of insulin secreting cells from nitric oxide induced cellular damage by crosslinked hemoglobin. *Biomaterials* **25**, 843–50.

  The ability of cross-linked hemoglobin (Hb-C) to mediate the effects of NO-induced stress on rat Islets of Langerhans cells and the rat insulinoma cell line (RINm5F) was evaluated. The rat cells were treated with the nitric oxide donor S-nitroso-N-acetylpenicillamine (SNAP). Twenty-four hours after SNAP treatment, the Griess Reagent System was used to determine levels of nitric oxide production. The DeadEnd™ Colorimetric TUNEL System was used to measure the effect of NO with or without Hb-C. In addition, cell viability was assessed using the CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay.

- Campos-Neto, A. *et al*. (1998) CD40 ligand is not essential for the development of cell-mediated immunity and resistance to *Mycobacterium tuberculosis*. *J. Immunol*. **160**, 2037–41.

  These authors used the Griess Reagent System to measure nitric oxide (NO) production in murine spleen cells.

For additional peer-reviewed articles that cite use of the Greiss Reagent System, visit: **www.promega.com/citations**

2. Product Components

Product	Size	Cat.#
Griess Reagent System	1,000 reactions	G2930

Includes:

- 50ml Sulfanilamide Solution (2 × 25ml)
- 50ml NED Solution (2 × 25ml)
- 1ml Nitrite Standard (0.1M Sodium Nitrite)

Storage Conditions: Store components at 4°C, protected from light. Return solutions to 4°C promptly after use. Store components separately; the shelf life is decreased substantially when the reagents are stored as a single, mixed solution.

The NED Solution may change color if it is not stored protected from light. However, this color change does not significantly affect product performance.

3. General Considerations

Sulfanilamide and NED compete for nitrite in the Griess reaction; thus greater sensitivity is achieved when the two components are added sequentially (4). Add the Sulfanilamide Solution to the sample first, incubate for 5–10 minutes, then add the NED Solution.

To ensure accurate NO_2^- quantitation, prepare a reference curve with the Nitrite Standard **for each assay**, using the same matrix or buffer used for experimental samples (Section 4.A). Due to substances that interfere with the Griess reaction, different levels of sensitivity may be achieved in different buffers or matrices. See Figure 2 for a series of representative reference curves for the Nitrite Standard in various matrices.

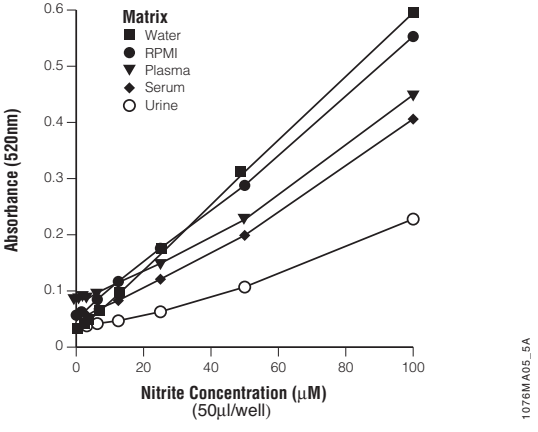

Figure 2. Representative Nitrite Standard reference curves in various matrices. Assays were performed as described in Section 4 using the Nitrite Standard in the following undiluted matrices: water, RPMI 1640 containing 15% serum and 5.3mg/L phenol red, bovine plasma, bovine calf serum and human urine.

4. Protocol for Determining Nitrite Concentration

Materials to Be Supplied by the User

- reagent reservoirs and multichannel pipettor
- 96-well flat-bottom enzymatic assay plate
- plate reader with 520–550nm filter

4.A. Preparation of a Nitrite Standard Reference Curve

A Nitrite Standard reference curve must be prepared **for each assay** for accurate quantitation of NO_2^- levels in experimental samples. Prepare reference curve(s) in the same matrix or buffer used for experimental samples.

1. Prepare 1ml of a 100µM nitrite solution by diluting the provided 0.1M Nitrite Standard 1:1,000 in the matrix or buffer used for the experimental samples.
2. Designate 3 columns (24 wells) in the 96-well plate for the Nitrite Standard reference curve (Figure 3). Dispense 50µl of the appropriate matrix or buffer into the wells in rows B–H.
3. Add 100µl of the 100µM nitrite solution to the remaining 3 wells in row A.
4. Immediately perform 6 serial twofold dilutions (50µl/well) in triplicate down the plate to generate the Nitrite Standard reference curve (100, 50, 25, 12.5, 6.25, 3.13 and 1.56µM), discarding 50µl from the 1.56µM set of wells. Do not add any nitrite solution to the last set of wells (0µM).

   Note: The final volume in each well is 50µl, and the nitrite concentration range is 0–100µM.

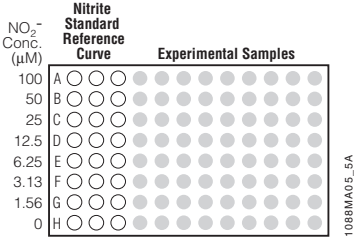

Figure 3. Suggested plate format for the Nitrite Standard reference curve.

4.B. Nitrite Measurement (Griess Reaction)

1. Allow the Sulfanilamide Solution and NED Solution to equilibrate to room temperature (15–30 minutes).
2. Add 50µl of each experimental sample to wells in duplicate or triplicate.
3. Using a multichannel pipettor, dispense 50µl of the Sulfanilamide Solution to all experimental samples and wells containing the dilution series for the Nitrite Standard reference curve.
4. Incubate 5–10 minutes at room temperature, protected from light.
5. Using a multichannel pipettor, dispense 50µl of the NED Solution to all wells.
6. Incubate at room temperature for 5–10 minutes, protected from light. A purple/magenta color will begin to form immediately.
7. Measure absorbance within 30 minutes in a plate reader with a filter between 520nm and 550nm. See Figure 4 for an absorbance spectrum of the colored azo compound.

Measure absorbance within 30 minutes. Color may fade after this time.

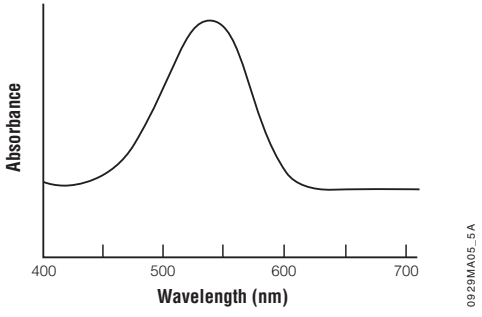

Figure 4. Absorbance spectrum of the colored azo compound.

4.C. Determination of Nitrite Concentrations in Experimental Samples

1. To generate a Nitrite Standard reference curve, plot the average absorbance value of each concentration of the Nitrite Standard as a function of "Y" with nitrite concentration as a function of "X".
2. Determine average absorbance value of each experimental sample. Determine its concentration by comparison to the Nitrite Standard reference curve.

5. Composition of Solutions

NED Solution

(0.1% N-1-napthylethylenediamine dihydrochloride in water)

Sulfanilamide Solution

(1% sulfanilamide in 5% phosphoric acid)

Nitrite Standard

(0.1M sodium nitrite in water)

6. Related Products

Product	Size	Cat.#
CytoTox-ONE™ Homogeneous	200–800 assays	G7890
Membrane Integrity Assay	1,000 assays	G7891
CytoTox 96® Non-Radioactive Cytotoxicity Assay*	1,000 assays	G1780

*For Laboratory Use.

7. References

1. Bredt, D.S. and Snyder, S.H. (1994) Nitric oxide: A physiologic messenger molecule. *Annu. Rev. Biochem.* **63**, 175–95.
2. Dawson, T.M. and Dawson, V.L. (1995) Nitric oxide: Actions and pathological roles. *The Neuroscientist* **1**, 7–18.
3. Griess, P. (1879) Bemerkungen zu der abhandlung der H.H. Weselsky und Benedikt "Ueber einige azoverbindungen." *Chem. Ber.* **12**, 426–8.
4. Fiddler, R.M. (1977) Collaborative study of modified AOAC method of analysis for nitrite in meat and meat products. *J. AOAC* **60**, 594–9.

Promega Corporation · 2800 Woods Hollow Road · Madison, WI 53711-5399 USA
Toll Free in USA 800-356-9526 · Phone 608-274-4330 · Fax 608-277-2516 · **www.promega.com**
Printed in USA. Part# TB229
Revised 6/09