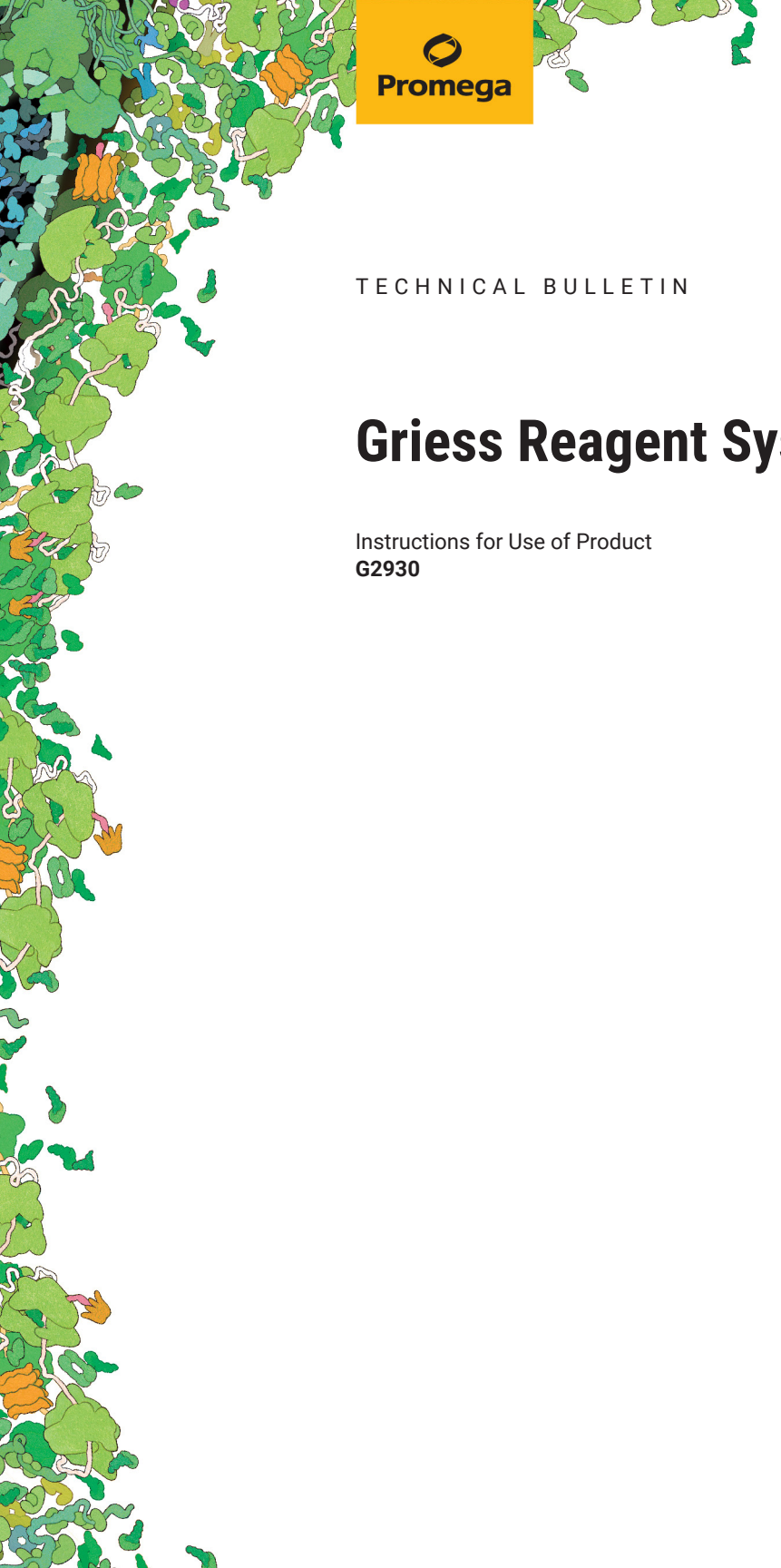

TECHNICAL BULLETIN

Griess Reagent System

Instructions for Use of Product
G2930

Revised 10/24
TB229

Griess Reagent System

All technical literature is available at: www.promega.com/protocols/
Visit the website to verify that you are using the most current version of this Technical Bulletin.
Email Promega Technical Services if you have questions on use of this system: 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.

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

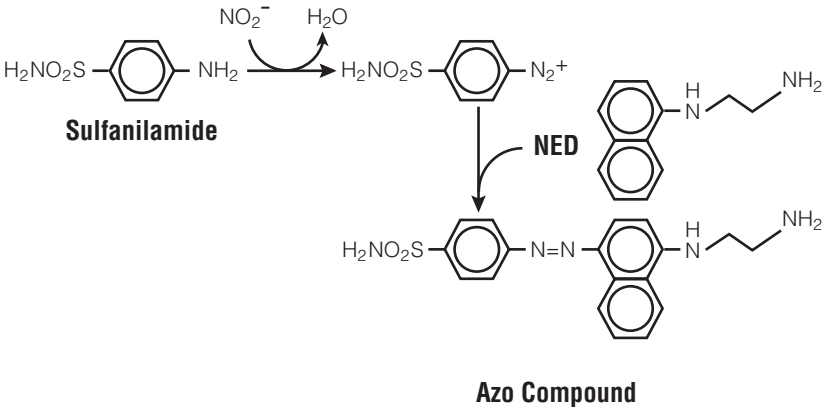

Figure 1. Chemical reactions involved in the measurement of NO_2^- using the Griess Reagent System.

2. Product Components and Storage Conditions

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 +2°C to +10°C, protected from light. Return solutions to +2°C to +10°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.

3. General Considerations (continued)

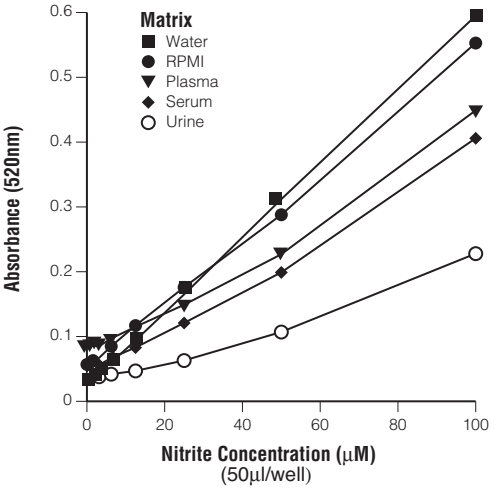

Figure 2. Representative Nitrite Standard reference curves in various matrices. Assays were performed as described in Section 4 using the Nitrite Standard in the following undiluted matrices: water, RPMI 1640 containing 15% serum and 5.3mg/L phenol red, bovine plasma, bovine calf serum and human urine.

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.

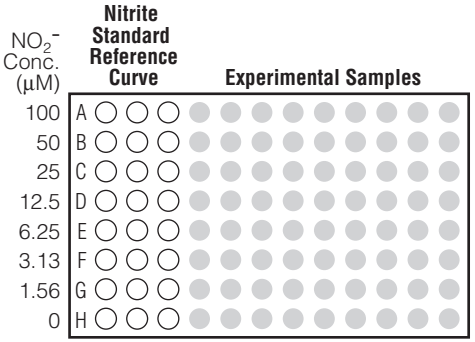

Figure 3. Suggested plate format for the Nitrite Standard reference curve.

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 for wavelengths 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.

4.B. Nitrite Measurement (Griess Reaction; continued)

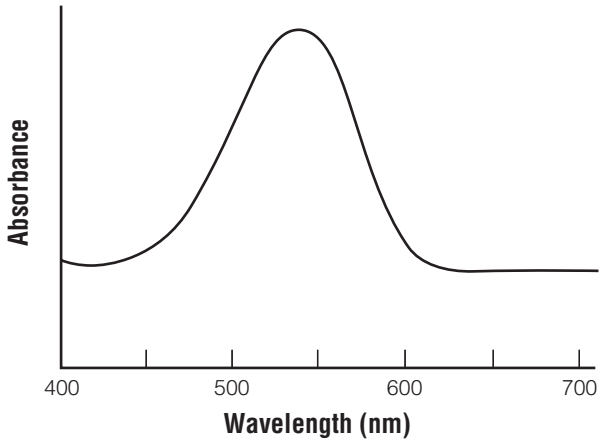

Figure 4. Absorbance spectrum of the colored azo compound.

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. 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.

7. Related Products

Oxidative Stress Assays

Product	Size	Cat.#
GSH-Glo™ Glutathione Assay	10ml	V6911
GSH/GSSG-Glo™ Assay	10ml	V6611
NAD/NADH-Glo™ Assay	10ml	G9071
NADP/NADPH-Glo™ Assay	10ml	G9081
Mitochondrial ToxGlo™ Assay	10ml	G8000

For Research Use Only. Not for Use in Diagnostic Procedures.
Additional sizes available.

Cell Viability, Cytotoxicity and Apoptosis Assays

Product	Size	Cat.#
CytoTox-ONE™ Homogeneous Membrane Integrity Assay	200–800 assays	G7890
	1,000 assays	G7891
CytoTox 96® Non-Radioactive Cytotoxicity Assay	1,000 assays	G1780
CellTiter-Glo® 2.0 Cell Viability Assay	10ml	G9241
CellTiter-Glo® 3D Cell Viability Assay	10ml	G9681
CellTiter-Fluor™ Cell Viability Assay	10ml	G6080
RealTime-Glo™ MT Cell Viability Assay	100 assays	G9711
CellTox™ Green Cytotoxicity Assay	10ml	G8741
LDH-Glo™ Cytotoxicity Assay	10ml	J2380
Caspase-Glo® 3/7 Assay System	2.5ml	G8090
RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay	100 assays	JA1011

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Additional sizes available.

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7. Related Products (continued)

Detection Instruments

Product	Size	Cat.#
GloMax® Discover System	1 each	GM3000
GloMax® Explorer System	1 each	GM3500

8. Summary of Changes

The following changes were made to the 10/24 revision of this document:

1. Contents moved to latest TB template, cover image and fonts were updated.
2. Removed citations list in Section 1. Find citations by searching at: **www.promega.com/citations/**
3. Updated Section 2 heading and storage temperatures in Section 2 to reflect current styles.
4. Updated Section 7, Related Products.