#13547 Store at 4°C

Griess Reagent Nitrite Measurement Kit

1 Kit
(500 assays)

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

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For Research Use Only. Not For Use In Diagnostic Procedures.

Description: The Griess Reagent Nitrite Measurement Kit can be used to indirectly detect nitric oxide (NO) through the measurement one of its stable oxidation products, nitrite. Nitrite reacts with sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride (NED) to yield a pink azo dye. The azo dye produced in this assay can be measured spectrophotometrically using its absorbance at 550 nM.

Background: Nitric Oxide (NO) is a pleiotropic signaling molecule that plays key roles in multiple cells types and tissues. NO is generated by three distinct nitric oxide synthase (NOS) enzymes that catalyze the oxidation of L-arginine into L-citrulline and NO (1). Two of these enzymes, endothelial NOS (eNOS) and neuronal NOS (nNOS), are constitutively expressed in many cell types (1). Inducible NOS (iNOS) is transcriptionally regulated and is expressed in response to inflammatory stimuli, such as pro-inflammatory cytokines (1). NO functions as a potent vasodialator and neurotransmitter (1). In the context of cancer, NO may function as either a tumor suppressor or tumor promoter (2). High levels of NO produced by macrophages are cytotoxic (2). In contrast, research studies have shown that lower levels of NO may contribute to tumorigenesis through promoting angiogenesis (2).

Specificity/Sensitivity: The Griess Reagent Nitrite Measurement Kit detects nitrite levels within a given sample. Detection of micromolar amounts of nitrite is possible using the Griess assay, with the detection range dependent upon the matrix or buffer used in the assay. An estimate of total NO produced requires determination of both nitrite and nitrate levels. As the kit measures only nitrite levels, nitrate should initially be reduced using a nitrate reductase.

Background References:

(1) Hill, B.G. et al. (2010) *J Biol Chem* 285, 19699-704.

(2) Rahat, M.A. and Hemmerlein, B. (2013) *Front Physiol* 4, 144.

Products Included	Product #	Quantity
0.1 M Nitrite Standard	13549	1 mL
Reagent A: Sulfanilamide Solution	13424	25 mL
Reagent B: NED Solution	13371	25 mL

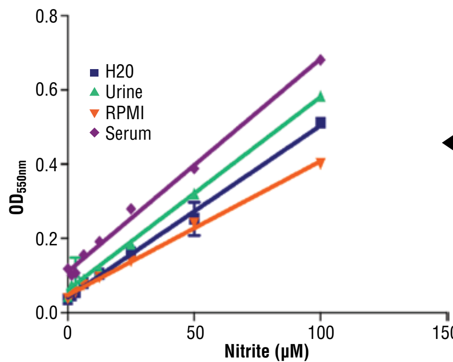

Figure 1: Representative standard curves in different assay matrices. Nitrite standard curves were generated in water, RPMI 10% FBS, serum, or urine.

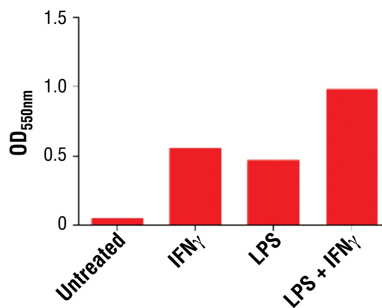

Figure 2: Detection of nitrite in supernatants of RAW 264.7 cells, untreated or treated with Mouse Interferon-γ (mIFN-γ) #5222 (100 ng/ml), LPS (10 ng/ml) or both. After 48 hours, cell culture supernatants were harvested and assayed for nitrite using the Griess Reagent Nitrite Measurement Kit.

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Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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Griess Reagent Nitrite Measurement Kit Protocol

A Solutions and Reagents

1. **Reagent A: Sulfanilamide Solution:** Bring to room temperature before use.
2. **Reagent B: NED Solution:** Bring to room temperature before use.
3. Prepare **Griess Reagent working solution** by mixing equal volumes of Reagent A: Sulfanilamide Solution and Reagent B: NED Solution.
4. **0.1 M Nitrite Standard:** Bring to room temperature before use. Prepare a 100 μM working nitrite standard by diluting the 0.1 M Nitrite Standard 1:1000 in the same matrix/buffer used for unknown samples. Perform serial two-fold dilutions of the 100 μM working nitrite standard solution. The final concentrations of nitrite in the standard curve should be as follows:
   100, 50, 25,12.5, 6.25, 3.13, and 1.56 μM. The blank should also be prepared in the same matrix/buffer.

A Test Procedure

1. Add 100 μl of sample or nitrite standard to each well of a microtiter plate.
2. Add 100 μl of Griess Reagent working solution per well. The solution will turn a pinkish color in the presence of nitrite.
3. Measure absorbance at 550 nM.
4. To quantify the amount of nitrite in unknown samples, plot the average absorbance against each respective concentration in the standard curve in an X vs. Y plot. Extrapolate the concentration of unknown samples from a linear curve.

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