Revised: April 1, 2020

Product Information

Griess Reagent Kit

Catalog Number: 30100

Kit Contents

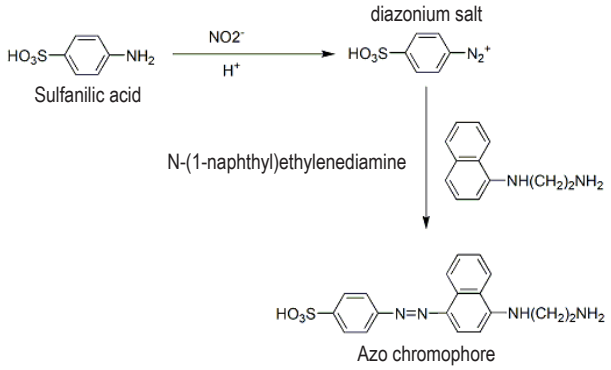
Component	Size
30100A: Griess Reagent (0.5% N-(1-naphthyl)ethylenediamine dihydrochloride, 0.5% sulfanilic acid, 2.5% phosphoric acid	50 mL
30100B: Nitrite Standard Solution (1 mM sodium nitrite)	1 mL

Storage and Handling

Store at 4°C and protected from light. Before using Griess reagent, warm it to room temperature and inspect the solution carefully for any precipitation. Any precipitates should go back into solution as it is warmed to room temperature. Product is stable for at least 12 months from date of receipt when stored as recommended. The Nitrite Standard Solution contains 1 mM sodium nitrite which is a potential mutagen. The toxicities of sulfanilic acid and N-(1-naphthyl)ethylenediamine are unknown.

Product Description

Griess reagent is used to detect nitrite photometrically. The reagent contains two chemicals, sulfanilic acid and N-(1-naphthyl)ethylenediamine. Under acidic conditions sulfanilic acid is converted by nitrite to a diazonium salt, which readily couples with N-(1-naphthyl)ethylenediamine to form a highly colored azo dye that can be detected at 548 nm:



Nitrite is often measured in samples as an indicator of nitric oxide (NO) without further sample treatment. In order to measure total NO indirectly from nitrite, nitrate must be reduced to nitrite enzymatically using nitrate reductase (not provided) before performing the assay so that the total amount of nitrite can be measured.[1-2] See "Nitrate Reduction Reaction" section under "Experimental Protocols".

References

1) *Methods Enzymol.* 268, 237 (1996); 2) *Ann Clin Biochem.* 34, 193 (1997); 3) *Anal. Biochem.* 126, 131 (1982); 4) *Proc. Natl. Acad. Sci. USA* 84, 9265 (1987); 5) *Biochem. Biophys. Res. Commun.* 161, 420 (1989); 6) *J. Exp. Med.* 169, 1543 (1989); 7) *Cell* 78, 919(1994); 8) *Science* 257, 494 (1992).

Experimental Protocols

Notes:

- Nitrite concentrations in the samples should be within the linear range of the assay (~1-100 uM).
- Nitrate formed from NO oxidation must be converted to nitrite for the analysis. Enzymatic reduction of nitrate to nitrite can be carried out using nitrate reductase. Methods for in-line reduction of nitrate to nitrite for automated nitrate analysis have been reported.[1-3] See "Nitrate Reduction Reaction" section for a detailed protocol.
- Preparation of biological samples for NO/nitrite analysis generally involves preparing a supernatant from a centrifuged cell lysate or collecting tissue perfusate as described in the literature.[4-6]

Calibration

1. Prepare sodium nitrite solutions with concentrations between 1-100 uM by diluting the nitrite standard solution with deionized water. Prepare enough of each standard to test in triplicate.

   Note: We recommend 6 serial two-fold dilutions of 100 uM, 50 uM, 25 uM, 12.5 uM, 6.25 uM, 3.13 uM and 1.56 uM.

2. Measure the absorbance of each standard as described in the spectrophotometry or microplate assay protocols below (300 uL for the cuvette assay or 150 uL for the microplate assay).

   Note: If you plan to measure total NO indirectly from nitrite, proceed to the nitrate reduction reaction protocol below.

3. Plot a standard curve of nitrite concentration vs. absorbance. Read nitrite concentrations corresponding to the absorbance of the samples.

Nitrate Reduction Reaction

The following protocol for nitrate reduction has been adapted from literature.[2]

1. Materials required but not provided

- Nitrate Reductase ≥300 U/g (Sigma catalog no. N7265)
- L-Glutamic Dehydrogenase from bovine liver ≥20 U/mg (Sigma catalog no. G7882)
- β-NADPH (Sigma catalog no. N1630)
- α-Ketoglutaric acid
- Ammonium chloride (NH_4Cl)
- 10X Phosphate-Buffered Saline (Biotium catalog no. 22020)

2. Nitrate Reduction Reaction Protocol

2.1 Add 1X PBS containing nitrate reductase and β-NADPH to a final concentration of 300 U/L and 25 uM, respectively.

2.2 Incubate your reactions at room temperature for 3 hours.

2.3 Consume excess β-NADPH by adding final concentrations of 500 U/L L-glutamic dehydrogenase, 4 mM α-ketoglutaric acid, and 100 mM NH_4Cl in 1X PBS.

2.4 Incubate reactions for 10 minutes at 37°C.

2.5 Proceed to adding Griess reagent and measuring absorbance as described in the spectrophotometry or microplate assay protocols on p.2.

Spectrophotometry

1. Combine the following in a cuvette with 1 cm path length:

   100 uL Griess reagent
   300 uL test sample
   2.6 mL deionized water

2. Incubate the mixture for ~30 minutes at room temperature.
3. Prepare a reference sample by adding 100 uL Griess reagent and 2.9 mL deionized water.
4. Measure the absorbance of the nitrite-containing sample at 548 nm relative to the reference sample.
5. Convert the optical density reading to nitrite concentrations as described above under Calibration.

Microplate Assay

1. In a microplate with a capacity of at least 300 uL/well, mix the following in each well:

   20 uL Griess reagent
   150 uL test sample
   130 uL deionized water

2. Incubate the mixture for ~30 minutes at room temperature.
3. Prepare a reference sample by mixing 20 uL Griess reagent and 280 uL deionized water.
4. Measure the absorbance of the nitrite-containing samples relative to the reference sample. For best results, measurements should be made at 548 nm. Other wavelengths in the range of 520-590 nm can also be used if the 548 nm wavelength is not available on your instrument.

Related Products

Catalog number	Product
00222	SNAP (S-Nitroso-N-acetylpenicillamine), 50 mg
00224	Spermine NONOate, 25 mg
00225	DEA-NONOate, 25 mg
00239	Nitric Oxide Generation Kit, 1 set
00240	7-Nitroindazole (7-NI), 50 mg
00249	NOS Inhibitor Kit, 1 kit
00300	DAA (1,2-Diaminoanthraquinone), 10 mg
00301	2,3-Diaminoapthalene, 100 mg
10055	Dihydrorhodamine 123, 10 mg
10057	Dihydroethidium (Hydroethidium), 25 mg
10058	H2DCFDA (2',7'-Dichlorodihydrofluorescein diacetate), 100 mg
22020	10X Phosphate-Buffered Saline (PBS), 4L
41024-4L	Water, Ultrapure Molecular Biology Grade, 4L

Please visit our website at www.biotium.com for information on our life science research products, including other dyes and probes for detecting nitric oxide (NO) or reactive oxygen species in cells. Also see our environmentally friendly EvaGreen® qPCR master mixes, fluorescent CF® dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.