FT-14301A

Griess Reagent Kit

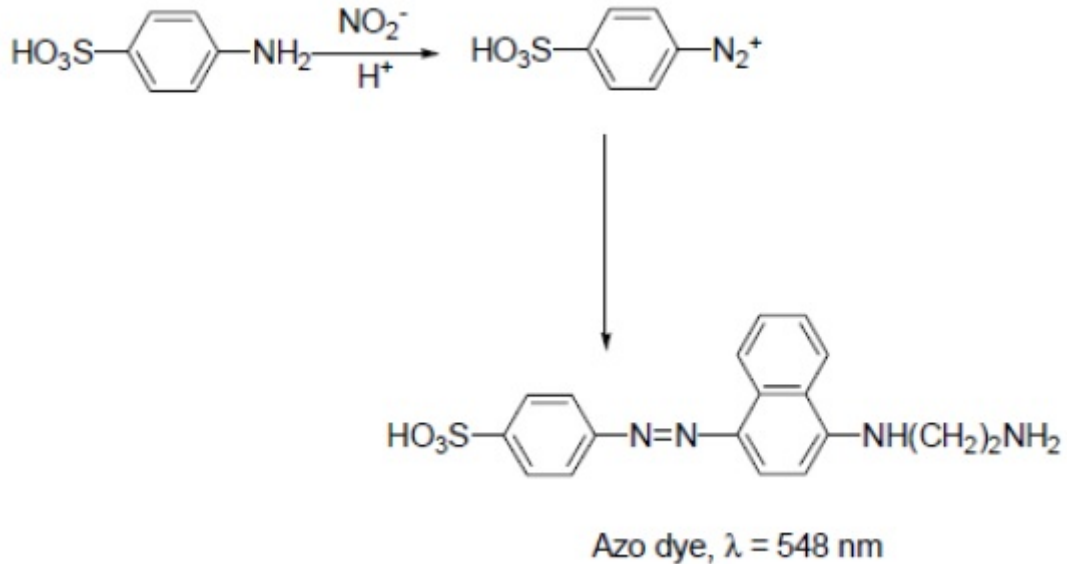
Product Description

Name :	**Griess Reagent Kit**
Catalog Number :	FP-14301A , 1kit (50 mL Griess Reagent and 1.0 mL Nitrite Standard Solution)
Components:	A) Griess Reagent containing: 1) 0.05% (0.5mg/mL) N-(1-naphthyl)ethylenediamine dihydrochloride; 2) 0.5% (5mg/mL) sulfanilic acid; and 3) 2.5% phosphoric acid. B) Nitrite Standard Solution containing 1.0 mM sodium nitrite in deionized water.
Absorption :	548 nm

Storage: Both Griess reagent and the nitrite standard solution should be stored refrigerated and protected from light. Before using Griess Reagent, warm it to room temperature and inspect the solution carefully for any precipitation. Any precipitates should usually redissolve when the solution is warmed up to room temperature

Introduction

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)etheylenediamine to form a highly colored azo dye that can be detected at 548 nm:



Azo dye, λ = 548 nm

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Under physiological conditions, NO is unstable and is rapidly oxidized to a mixture of nitrite and nitrate. In order to measure NO levels indirectly from nitrite, nitrate must be reduced to nitrite enzymatically using nitrate reductase before performing the assay so that the total amount of nitrite can be measured.

Directions for use

Guidelines for use

NOTES:
A) Nitrite concentrations in the samples should be within the linear range of the assay (~1-100 µM).
B) Nitrate formed from NO oxidation must be quantitatively 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]
C) 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 literatures.[2-4]

1. Spectrophotometry:
    1.1 Combine the following in a cuvette with 1 cm pathlength:
        100 µL Griess Reagent
        300 µL nitrite-containing sample (see Notes)
        2.6 mL deionized water
    1.2 Incubate the mixture for ~30 minutes at room temperature.
    1.3 Prepare a reference sample by adding 100 µL Griess Reagent and 2.9 mL deionized water.
    1.4 Measure the absorbance of the nitrite-containing sample at 548 nm relative to the reference sample.
    1.5 Convert the optical density reading to nitrite concentrations as described below under Calibration.
2. Microplate Assay:
    2.1 In a microplate with a capacity of at least 300 µL/well, mix the following in each well:
        20 µL Griess Reagent
        150 µL the nitrite-containing sample (See Notes)
        130 µL deionized water
    2.2 Incubate the mixture for ~30 minutes at room temperature.
    2.3 Prepare a photometric reference sample by mixing 20µL Griess Reagent and 280µL deionized water.
    2.4 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.
3. Calibration:
    3.1 Prepare sodium nitrite solution with concentrations between 1-100 µM by diluting the nitrite standard solution with deionized water.
    3.2 Prepare samples and measure the absorbance as describes above, using the standard nitrite solutions (300µL for the cuvette assay or 150µL for the microplate assay) in place of the experimental samples.
    3.3 Plot a standard curve of nitrite concentration vs. absorbance. Read nitrite concentrations corresponding to the absorbance of the samples.

References

1) Anal. Biochem. 126, 131 (1982); 2) Proc. Natl. Acad. Sci. USA 84, 9265 (1987); 3) Biochem. Biophys. Res. Commun. 161, 420 (1989); 4) J. Exp. Med. 169, 1543 (1989); 5) Cell 78, 919(1994); 6) Science 257, 494 (1992).

TOXICITY:

Sodium nitrite is a potential mutagen. The toxicities of sulfanilic acid and N-(1-naphthyl)ethylenediamine are unknown.

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Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com.
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06

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