Product Information

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Griess reagent (modified)

Catalog Number **G4410**
Store at Room Temperature

TECHNICAL BULLETIN

Product Description

Griess first described a colorimetric assay to measure the levels of nitrite (NO_2^-) in aqueous solutions over 100 years ago.[1] Modifications to the Griess method have been published in more recent years.[2,3] A fluorometric assay procedure that is 50-100 times as sensitive as the Griess colorimetric assay has been published.[4]

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

1× Griess Reagent – Add 250 ml of water to the bottle. Mix by inversion of the bottle for about five minutes. Note: Distilled water, water which has been treated with a mixed bed ion exchange resin, or ultrapure (17 MΩ·cm or equivalent) water are considered nitrite-free water and suitable for use. Nitrite-free water should be stored in glass vessels to prevent any possible leaching of NO_2^- compounds from the container.

Storage/Stability

Store the powdered product at room temperature.

The prepared 1× Griess Reagent may be stored for up to three months at room temperature.

Procedure

1. Mix equal volumes of 1× Griess Reagent and sample (working range: 0.43–65 µmolar nitrite).
2. Read the absorbance at 540 nm after 15 minutes.

Notes: For blood samples, it has been reported that pyridine nucleotides like NADPH and NADH strongly inhibit the Griess reaction.[5] Also, the presence of heparin in plasma or lymph samples may produce precipitation upon addition of the Griess reagent. Therefore, heparin must be removed prior to the reaction. To remove heparin, add protamine sulfate, vortex, and incubate for 5 minutes at room temperature. Centrifuge the mixture at 10,000 rpm for 10 minutes to pellet the insoluble heparin-protamine complex. The 1× Griess Reagent is then added to the supernatant. One mg of protamine sulfate will neutralize ~90 USP units of bovine lung heparin or ~115 USP units of porcine heparin.

Control - If the 1× Griess Reagent mixed with nitrite-free water produces a solution with an absorbance higher than the 1× Griess Reagent alone, the false positive reading may be due to either nitrate impurities which may have leached from containers into the nitrite-free water (even if the water had been distilled and/or deionized), to the presence of reducing agents, or to the presence of metal ions which may have come from stainless steel containers in which the nitrite-free water had been stored.

References

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2. Green, L.C., *et al*., Analysis of nitrate, nitrite, and [^{15}N]nitrate in biological fluids. Anal. Biochem., **126(1)**, 131-138 (1982).
3. Pollock, J.S., *et al*., Purification and characterization of particulate endothelium-derived relaxing factor synthase from cultured and native bovine aortic endothelial cells. Proc. Nat. Acad. Sci. USA, **88(23)**, 10480-10484 (1991).
4. Misko, T.P., *et al*., A fluorometric assay for the measurement of nitrite in biological samples. Anal. Biochem., **214(1)**, 11-16 (1993).
5. Grisham, M. B., *et al*., Quantitation of nitrate and nitrite in extracellular fluids. Methods Enzymol., **268**, 237-246 (1996).

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