

## Griess Reagent Kit for Nitrite Determination (G-7921)

### Quick Facts

#### Storage upon receipt:

- 2–6°C
- Do not freeze
- Protect from light
- Protect from air

**Abs of reaction product:** 548 nm

**Precautions:** Component B contains 5% phosphoric acid; handle with care.

forming an azo dye that can be spectrophotometrically quantitated based on its absorbance at 548 nm.

### Materials

#### Reagents Supplied

- ***N*-(1-naphthyl)ethylenediamine dihydrochloride** (Component A), 25 mL of a 0.1% (1 mg/mL) solution, sealed under argon
- **Sulfanilic acid** (Component B), 25 mL of a 1% (10 mg/mL) solution in 5% phosphoric acid
- **Nitrite standard solution** (Component C), 1.0 mL of 1.0 mM sodium nitrite in deionized water

#### Storage and Handling

All components of this kit should be stored refrigerated at 2–6°C, protected from light. DO NOT FREEZE. We recommend that you take reasonable precautions to protect *N*-(1-naphthyl)ethylenediamine (Component A) from air oxidation. Sulfanilic acid (Component B) may come out of solution upon storage — the crystals will readily redissolve at room temperature.

**Caution:** Sulfanilic acid (Component B) is dissolved in a phosphoric acid solution. Handle and dispose of the undiluted reagent in accordance with standard laboratory procedures for using acids.

### Introduction

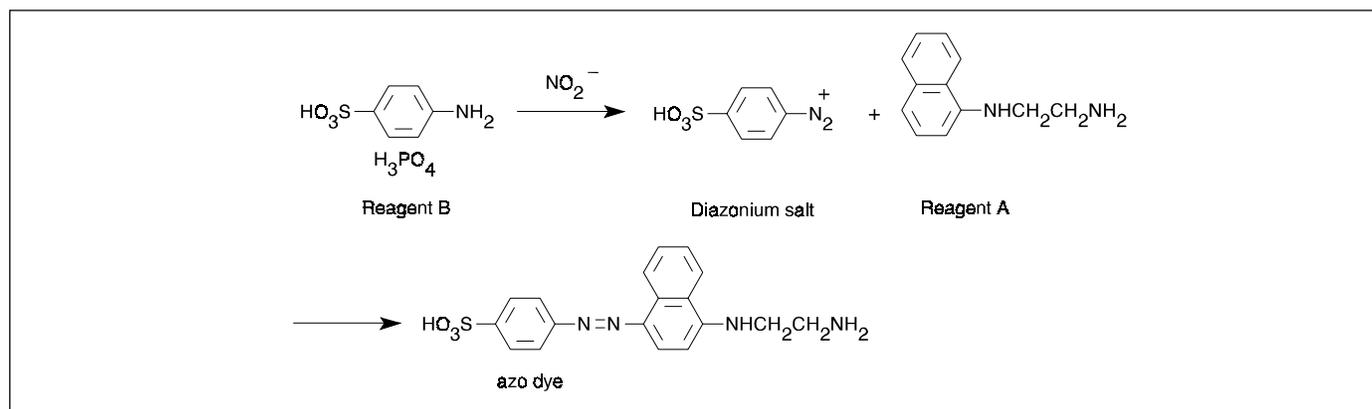
Nitric oxide (NO) is a molecular mediator of many physiological processes,<sup>1,2</sup> including vasodilation, inflammation, thrombosis, immunity and neurotransmission. A number of methods exist for measuring NO in biological systems.<sup>3</sup> One of these methods involves the use of the Griess diazotization reaction to spectrophotometrically detect nitrite formed by the spontaneous oxidation of NO under physiological conditions.<sup>4,7</sup> The detection limit for this method is 1.0 μM nitrite.<sup>5</sup> The Griess reaction can also be used to analyze nitrate via its catalytic reduction to nitrite.<sup>8</sup>

Our Griess Reagent Kit provides all the reagents required for nitrite quantitation in biological samples. Figure 1 shows the principle of the assay. Sulfanilic acid is quantitatively converted to a diazonium salt by reaction with nitrite in acid solution. The diazonium salt is then coupled to *N*-(1-naphthyl)ethylenediamine,

### Protocols

#### Preparation of the Griess Reagent

Mix together equal volumes of *N*-(1-naphthyl)ethylenediamine (Component A) and sulfanilic acid (Component B) to form the Griess Reagent. Prepare sufficient reagent for immediate experiments only (100 μL per spectrophotometer cuvette,



**Figure 1.** Principle of nitrite quantitation using the Griess reaction. Formation of the azo dye is detected via its absorbance at 548 nm.

20 µL per microplate well). Do not store the mixture for more than 8 hours.

### **Spectrophotometer Assay**

**1.1** Mix the following in a spectrophotometer cuvette (1 cm pathlength):

- 100 µL of Griess Reagent
- 300 µL of the nitrite-containing sample (see notes **A** and **B**)
- 2.6 mL of deionized water

Nitrite concentrations in the samples should fall within the linear range of the assay (approximately 1–100 µM).

**1.2** Incubate the mixture for 30 minutes at room temperature.

**1.3** Prepare a photometric reference sample by mixing 100 µL of Griess Reagent (above) and 2.9 mL of deionized water.

**1.4** Measure the absorbance of the nitrite-containing sample at 548 nm relative to the reference sample.

**1.5** Convert absorbance readings to nitrite concentrations as described in *Calibration*, below.

### **Microplate Assay**

**2.1** In a microplate (sample capacity at least 300 µL per well), mix the following in each well:

- 20 µL of Griess Reagent
- 150 µL of the nitrite-containing sample (see notes **A** and **B**)
- 130 µL of deionized water

**2.2** Incubate the mixture for 30 minutes at room temperature.

**2.3** Prepare a photometric reference sample by mixing 20 µL of Griess Reagent and 280 µL of deionized water.

**2.4** Measure the absorbance of the nitrite-containing samples relative to the reference sample in a spectrophotometric

microplate reader. The optimum measurement wavelength is 548 nm. Other wavelengths in the range 520–590 nm can be used if 548 nm is not available on your instrument.

**2.5** Convert absorbance readings to nitrite concentrations as described in *Calibration*, below.

### **Calibration**

**3.1** Prepare sodium nitrite solutions with concentrations between 1–100 µM by diluting the nitrite standard solution (Component C) with deionized water.

**3.2** Prepare samples and make absorbance measurements as described above, using the standard nitrite solutions (300 µL for the cuvette assay or 150 µL for the microplate assay) in place of the experimental samples.

**3.3** Plot a standard curve of nitrite concentration (x-axis) against absorbance (y-axis). Read nitrite concentrations corresponding to the absorbance of experimental samples from the standard plot.

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### **Notes**

[A] Nitrates must be quantitatively converted to nitrites for analysis. Enzymatic reduction of nitrate to nitrite can be carried out using nitrate reductase. Methods for in-line reduction during automated analysis of nitrates are described in the literature;<sup>8</sup> for example, samples can be passed through a column containing copper-plated cadmium filings to convert nitrates to nitrites.

[B] Preparation of biological samples for analysis generally involves preparing a supernatant from a centrifuged cell lysate or collecting tissue perfusate. Consult literature references for specific protocols.<sup>4,6,7</sup> Analysis of nitrites produced in response to physiological stimuli requires careful control measurements to account for nitrite from metabolic or dietary sources.

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### **References**

1. Cell 78, 919 (1994); 2. Science 257, 494 (1992); 3. FASEB J 7, 349 (1993); 4. Proc Natl Acad Sci USA 84, 9265 (1987); 5. J Pharmacol Exp Ther 252, 922 (1990); 6. Biochem Biophys Res Commun 161, 420 (1989); 7. J Exp Med 169, 1543 (1989); 8. Anal Biochem 126, 131 (1982).

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