# Griess Reagent Kit, for nitrite quantitation

Catalog Numbers G7921

Pub. No. MAN0001938 Rev. B

**WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

## Product description

Nitric oxide (NO) is a molecular mediator of many physiological processes, including vasodilation, inflammation, thrombosis, immunity and neurotransmission. A number of methods exist for measuring NO in biological systems. 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. The detection limit for this method is 1.0 µM of nitrite. The Griess reaction can also be used to analyze nitrate via its catalytic reduction to nitrite.

The Griess Reagent Kit (Cat.No. G7921) provides all the reagents required for nitrite quantitation in biological samples. The following image 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, forming an azo dye that can be spectrophotometrically quantitated based on its absorbance at 548 nm.

**Note:** Formation of the azo dye is detected via its absorbance at 548 nm.

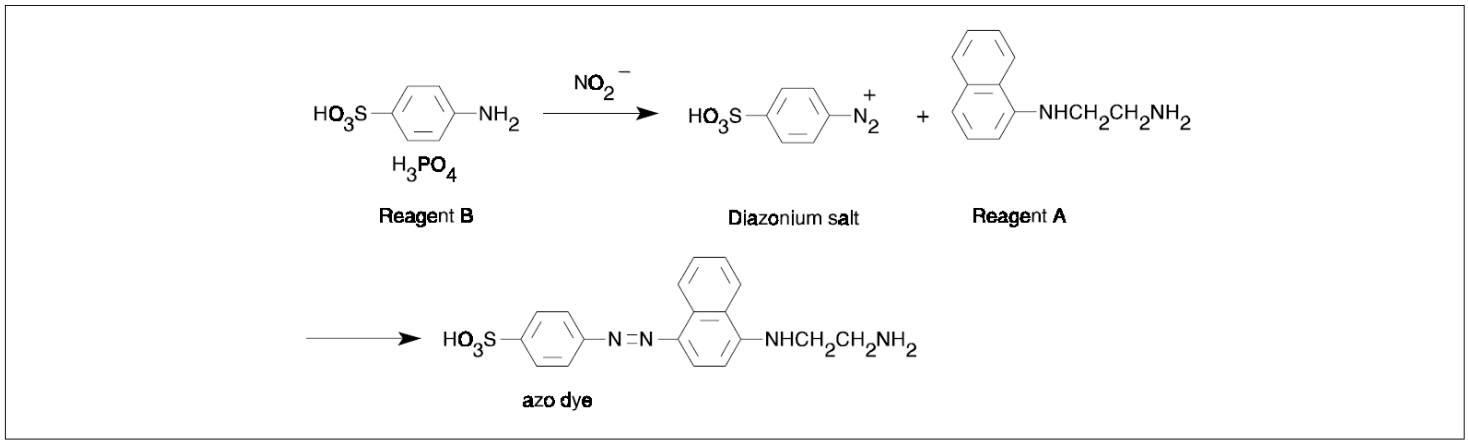


**Figure 1 Principle of nitrite quantitation using the Griess Reaction.**

## Contents and storage

**Table 1 Griess Reagent Kit, for nitrite quantitation (Cat.No. G7921)**

| Item | Amount | Concentration | Storage |
|---|---|---|---|
| *N*-(1-naphthyl)ethylenediamine dihydrochloride (Component A) [1] | 25 mL | 0.1% (1 mg/mL) solution, sealed under argon | • 2–8°C<br>• Do not freeze<br>• Protect from light<br>• Protect from air |
| **Sulfanilic acid** (Component B) [2] | 25 mL | 1% (10 mg/mL) solution in 5% phosphoric acid | |
| **Nitrite standard solution** (Component C) | 1.0 mL | 1.0 mM sodium nitrite in deionized water | |

[1] Use precautions to protect N-(1-naphthyl)-ethylenediamine (Component A) from air oxidation.
[2] Large crystals can form in the solution and can take up to one week to dissolve at room temperature. However, complete dissolution is not required for using the kit.

## Preparation

1. Mix together equal volumes of *N*-(1-naphthyl)ethylenediamine (Component A) and sulfanilic acid (Component B) to form the Griess Reagent.
2. Prepare sufficient reagent for immediate experiments only (100 µL per spectrophotometer cuvette or 20 µL per microplate well).
3. Do not store the mixture for more than 8 hours.

## Perform a spectrophotometer assay

1. Mix the following in a spectrophotometer cuvette (1–cm path length):
   - 2.6 mL of deionized water
   - 100 µL of Griess Reagent
   - 300 µL of the nitrite-containing sample

     **Note:** 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; for example, samples can be passed through a column containing copper-plated cadmium filings to convert nitrates to nitrites.

     **Note:** 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. Analysis of nitrites produced in response to physiological stimuli requires careful control measurements to account for nitrite from metabolic or dietary sources.
2. Incubate the mixture for 30 minutes at room temperature.
3. Prepare a photometric reference sample by mixing 100 µL of Griess Reagent and 2.9 mL of deionized water.
4. Measure the absorbance of the nitrite-containing sample at 548 nm relative to the reference sample.
5. Convert absorbance readings to nitrite concentrations as described in *“Generate a standard curve to calibrate nitrite quantitation” on page 3*.

## Perform a microplate assay

1. In a microplate, mix the following in each well:
   - 130 µL of deionized water
   - 20 µL of Griess Reagent
   - 150 µL of the nitrite-containing sample standard nitrite solutions

     **Note:** 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; for example, samples can be passed through a column containing copper-plated cadmium filings to convert nitrates to nitrites.

     **Note:** 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. Analysis of nitrites produced in response to physiological stimuli requires careful control measurements to account for nitrite from metabolic or dietary sources.
2. Incubate the mixture for 30 minutes at room temperature.
3. Prepare a photometric reference sample by mixing 20 µL of Griess Reagent and 280 µL of deionized water.
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 of 520–590 nm can be used if 548 nm is not available on your instrument.
5. Convert absorbance readings to nitrite concentrations as described in *“Generate a standard curve to calibrate nitrite quantitation” on page 3*.

## Generate a standard curve to calibrate nitrite quantitation

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

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. 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.

## Limited product warranty

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**Revision history:** Pub. No. MAN0001938 B

| Revision | Date | Description |
|---|---|---|
| B | 20 December 2024 | The statement about crystal dissolution was revised for clarity.<br>The document was updated to the current template, with associated updates to the limited license information, warranty, trademarks, and logos. |
| A | 9 July 2003 | New document for product insert. |