# Product Information

## Griess Kit

Catalog Number: S6025

Product Size: 1 T

Contents:

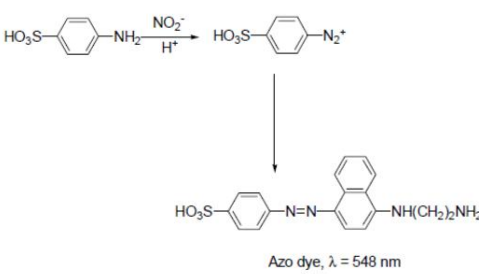
| Component | 50 T |
|---|---|
| A. Griess reagent: Contains 0.05% (0.5 mg/mL)N- (1-naphthyl) ethylenediamine dihydrochloride; 0.5% (5 mg/mL) sulfonic acid; 2.5% phosphoric acid | 50 mL |
| B. Nitrite standard solution (1.0 mM sodium nitrite dissolved in deionized water) | 1 mL |

## Storage

Griess reagent and nitrite standard solution should be refrigerated at 4℃ and protect from light. Before using the Griess reagent, restore it to room temperature and check the solution has precipitation or not. Any precipitates shall be re-dissolved when the solution is returned to room temperature.

## Description

Griess reagent can be used for determination of nitrite by spectrophotometry. The reagent contains two chemicals, sulfamic acid and N-(1-naphthyl) ethylenediamine. In acid condition, sulfonic acid is converted into diazonium salt by nitrite, which can form highly colored azo dye with N-(1-naphthyl) ethylenediamine, and the dye can be detected at 548 nm.




Azo dye, λ = 548 nm

Under physiological conditions, NO is unstable and will be rapidly oxidized into a mixture of nitrite and nitrate. In order to measure NO level indirectly according to nitrite, nitrate must be reduced to nitrite by nitrate reductase before measuring nitrate, so the total amount of nitrite can be measured.

## Protocol

**1. Spectrophotometry**

1.1 Add the following substances to a cuvette of 1 cm optical path length:

100 µL Griess reagent

300 µL nitrite samples (see remarks)

2.6 mL deionized water

**Remarks:**

1). The nitrite concentration in the sample should be within the linear range of determination (about 1-100 µM)

2). The nitrate formed by NO oxidation must be quantitatively converted into nitrite for analysis. Nitrate can be reduced to nitrite by nitrate reductase.

3). The preparation of biological samples for NO/nitrite analysis is usually to collect supernatant from centrifuged cell



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lysate or tissue perfusion solution.

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

1.3 Mix 100 μL Griess reagent and 2.9 mL deionized water to prepare the reference sample.

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

1.5 Convert the optical density reading to nitrite concentration as described in the calibration(step 3) below.

**2. Microplate determination**

2.1 Add the following substances to a cuvette of 1 cm optical path length:

100 μL Griess reagent

300 μL nitrite samples (see remarks in step 1.1)

2.6 mL deionized water

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

2.3 Mix 20 μL Griess reagent and 280 μL deionized water to prepare the reference sample.

2.4 Measure absorbance of sample containing nitrite relative to reference sample. To get the best results, measurements should be at 548 nm. If there is no 548 nm wavelength on the instrument, other wavelengths in the 520-590 nm range can also be used for detection.

**3. Calibration**

3.1 Nitrite standard solution was diluted with deionized water to prepare sodium nitrite solution with a concentration of 1-100 μM.

3.2 Use standard nitrite solution (take 300 μL for cuvette determination or 150 μL for microplate determination) to prepare samples to replace the above experimental samples, and detect the absorbance.

3.3 Draw the standard curve of nitrite concentration and absorbance, and read the nitrite concentration corresponding to sample absorbance.

## Notes

1. This product has potential hazards, avoid long-term or repeated contact. Avoid entering eyes, skin or clothing.

2. For your safety and health, please wear lab coats and disposable gloves.



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