

# Product Information

## Nitric Oxide Assay Kit

Catalog Number: N6025

Product Size: 200T / 1000T ( 96-well plate )

Contents:

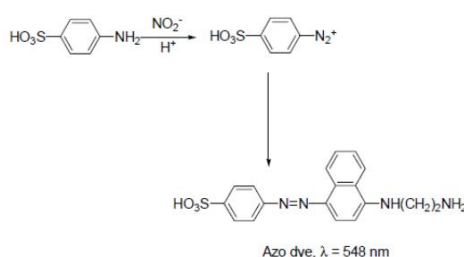
Component	200T	1000T
A. Griess Reagent I	10 mL	50 mL
B. Griess Reagent II	10 mL	50 mL
C. 1 M NaNO <sub>2</sub>	200 μL	1 mL

## Storage

Store at -20°C, protected from light.

## Description

Griess reagent can be used determination of nitrite by spectrophotometric. The reagent contains two chemicals, sulfonic acid and N-(1-naphthyl) ethylenediamine. Under acidic conditions, sulfonic acid is converted into diazonium salt by nitrite, which can form highly colored azo dye product with N-(1-naphthyl) ethylenediamine that absorbs visible light at 548 nm:



NO is unstable, can be oxidized to form nitrite and nitrate. Griess indirectly reflects the content of NO by detecting the content of nitrite.

## Protocol

1. Prepare Griess reagent I and II and return them to room temperature.

2. **Standard dilution:** dilute the standard NaNO<sub>2</sub> (1-100 μM) with the solution of the tested sample. The standard can be diluted into 1 μM, 10 μM, 20 μM, 40 μM, 80 μM, 100 μM, and add 100 μL standard per well . If the sample concentration is too low, the range of standard dilution (1 μM, 2 μM, 3 μM, 4 μM, 6 μM, 8 μM, 10 μM) can be appropriately reduced.

### 3. Sample testing

3.1 According to the total volume of 200 μL/well, 100 μL/well sample is added into 96-well plate; if the sample is the supernatant of culture medium, it can be directly sampled; if there is sediment, the supernatant should be centrifuged. If the sample is a cell or tissue, it can be rapidly freeze-thawed, and then centrifugally precipitated to obtain the supernatant. If the volume is less than 100 μL, it can be diluted with diH<sub>2</sub>O or 0.9% NaCl (the standard sample should also be diluted with distilled water or 0.9% NaCl).

3.2 Add 50 μL/well Griess reagent I.

3.3 Add 50 μL/well Griess reagent II.

3.4 The absorbance was measured at 540 nm.

If there is no 540 nm filter, 520-560 nm filter can be used. If there is no microplate or suitable filter, the concentration of NO in the sample can also be determined by visual colorimetry. A more precise concentration gradient is required for the standard



to be measured visually.

### Notes

1. Before using Griess reagent, return it to room temperature and check for precipitation in the solution. If Griess reagent I

contains precipitates, it can be placed in a 37°C water bath until the precipitate dissolves.

2. This product is potentially harmful. Avoid long-term or repeated contact. Avoid contact with eyes, skin or clothing.

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

