

# **Product Information**

## Nitric Oxide Assay Kit

Catalog Number: N6025

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

Contents:

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

### 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 548nm:

$$HO_3S$$
  $NO_2$   $HO_3S$   $NO_2$   $NO_2$ 

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  $\mu$ M) with the solution of the tested sample. The standard can be diluted into 1  $\mu$ M, 10  $\mu$ M, 20  $\mu$ M, 40  $\mu$ M, 80  $\mu$ M, 100 $\mu$ M, and add 100  $\mu$ l standard per well . If the sample concentration is too low, the range of standard dilution(1 $\mu$ M, 2  $\mu$ M, 3  $\mu$ M, 4  $\mu$ M, 6  $\mu$ M, 8  $\mu$ M, 10  $\mu$ M) can be appropriately reduced.

#### 3. Sample testing

- 3.1 According to the total volume of 200  $\mu$  L / well, 100  $\mu$  L / 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  $\mu$ L, 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).
- $3.2\,Add~50~\mu L$  / well Griess reagent I.
- 3.3 Add 50 µL / well Griess reagent I.
- 3.4 The absorbance was measured at 540nm.

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





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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 clothes and disposable gloves.