Colorimetric Nitric Oxide (NO) Assay Kit

Introduction

Nitric oxide (NO) is a key signaling molecule in living organisms, which is widely present in neurological, circulatory, digestive, respiratory, and genitourinary systems, and plays an important role in both physiological and pathological processes. NO can be oxidized to nitrite and nitrate. A common method to detect NO indirectly is to measure its stable oxidation product, nitrite.

Colorimetric Nitric Oxide (NO) Assay Kit is a kit for the determination of NO in samples such as cultured cells, tissues, serum, plasma, and urine by an optimized Griess Reagent. The principle is that the Griess Reagent can react with nitrite, and the resulting product has specific absorption at 540 nm. The absorbance is positively correlated with the NO content. The kit is optimized to detect down to 1 μM and has a good linearity in the range of 1-100 μM.

Components and Storage

Components	K2107- 2500 T
1M $NaNO_2$	1 mL
Griess Reagent A	25 mL
Griess Reagent B	25 mL
Store the kit at -20°C away from light, stable for 1 year.	

Protocol

1. **Dilution of the standard (1 M $NaNO_2$):** Perform a serial dilution of 1 M $NaNO_2$ with sterilized water for subsequent preparation of the standard curve. We recommend a serial dilution of 0, 1, 2, 5, 10, 20, 40, 60, 100 μM, at least 200 μL each.
2. **Preparation of the Griess Reagent:** Mix appropriate Griess Reagent A and Griess Reagent B at a ratio of 1:1 to make the Griess Reagent. Prepare sufficient reagent for immediate experiments. Pay attention to preparing fresh Griess Reagent every time. And the preparation process needs to be protected from light.

***Note:** Griess Reagent A and Griess Reagent B need to be warm to room temperature before use.

3. **Preparation of the sample:** The cell culture supernatant can be taken directly for detection. If there is precipitation, it is better to obtain a clear supernatant for detection after centrifugation. Cells or tissue samples

can be rapidly freeze-thaw lysed or lysed with lysis buffer (WB and IP cell lysis buffer is recommended), and then the supernatant for detection can be acquired by centrifugation.

***Note:** It is not recommended to use RIPA lysis buffer to lyse samples, as there may occur precipitate after using the RIPA lysis buffer and disturb the detection.

4. **Preparation of detection system:** Mixing each well in a microplate (sample capacity at least 300 μL) according to the following requirements, take care to not produce air bubbles when adding, and incubate at room temperature for 30 minutes after mixing. If the volume of the microplate used is smaller than this system, the amount of reagent used in the table below can be halved, i.e., the system can be reduced to 150 μL without affecting the experimental results.

Reagent	Volume
Sterilized water	130 μL
Sample/Serial dilution standards	150 μL
Griess Reagent	20 μL
Total	300 μL

5. **Detection:** After the incubation, measure the absorbance at 540 nm by the microplate reader, and the NO content in the sample can be estimated after the standard curve is obtained.

Note

1. If there is precipitation in Griess Reagent B after thawing, it can be shaken to promote dissolution, and it can be used normally after it is completely dissolved.
2. The solutions in this kit should not be exposed to air for a long time, otherwise it may be invalid.
3. If a lysis buffer is required to lyse cells, it is better to use WB and IP cell lysis buffer (Cat. No. K1123) rather than RIPA lysis buffer.
4. This product is harmful to the human body, please pay attention to protection when operating.
5. For your safety and health, please wear lab coats and gloves during the experiment.
6. For research use only. Not to be used in clinical diagnostic or clinical trials.

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