For research purpose only. Not for use in diagnostic procedures for clinical purposes. For IN VITRO USE ONLY.

ISO 9001/14001 Certified Company

Nitric Oxide (NO) Detection Kit

Cat. No. 21021 **500 - 1,000 assay**

DESCRIPTION

Nitric oixde (NO^-) is radical compound produced during the transformation of L-arginine to citrulline by nitric oxide synthase (NOS). Nitric oxide functions as the effecter molecule for macrophage-mediated cytotoxicity and has also been recognized to inhibit mitochondrial respiration and DNA synthesis. Nitric oxide recently raised a lot of interests as a suppressive factor that inhibits anti-tumor immune response. The nitric oxide detection kit from iNtRON, based on diazotization (Griess method) assay can detect *in vitro* NO^- concentration. The kit enables researchers to overcome difficulties in detecting NO^- concentration due to the short half life (about 5 seconds) of gaseous nitric oxide. The kit will accurately detect the concentration of NO^- by indirectly measuring nitrite (NO_2^-), which is by-product of nitric oxide transformation in living tissue. The kit is based on the colorimetric change, which occurs when naphthylethylenediamine is added to the by-product of reaction between sulfanilamide and nitrite. The limit of detection is 2.0μM nitrite in ultrapure distilled water using the recommended protocol.

STORAGE AND STABILITY

Store at 4°C, stable for 6 months.

CONTENTS

• N1 buffer (Substrate solution) : Sulfanilamide in the reaction buffer	50 ml
• N2 buffer (Coloring solution) : Naphthylethylenediamine in the stabilizer buffer	50 ml
• Nitrite (FW: 69.0) standard, 1mM	1 ml

TECHNICAL TIPS BEFORE USE

1. Store components at 4°C in their original plastic bottles. Please solutions back to 4°C promptly after use.
   When N1 buffer stores in a refrigerator, there could be little amount of sediments. In the presence of sediments, please dissolve the sediments with vortexing at RT
2. The greater sensitivity is achieved when the two buffers are added sequentially. Add the N1 buffer to the sample first, incubate for 5-10 min, then add the N2 buffer.
3. When collecting supernatant from the cultured cells, be careful not to include cell debris. The following steps are recommended to eliminate deviation. First, harvest cells by centrifuging. Second, perform a filtration using 0.22μm filter.
4. You may have to prepare a reference curve with the Nitrite standard solution to ensure accurate NO_2^- amount, using the same buffers or media used for experimental samples. The different levels of sensitivity may be achieved in different buffers or media.
5. The sample (or standard soln) volume of each well is 100μl, and the nitrite concentration range is 0-1,000μM (1mM). If the samples contain over 1mM of nitrite, the reaction color may initially become dark pink, but it will eventually turn to yellow as reaction is completed. In this case, you must dilute the samples with the same buffers or media.
6. In measurement of absorbance, you can use a plate reader with a filter between 520-560nm within 20-30min.

PROTOCOL I (For sample measurement)

1. Collect about 100μl of samples, and add the samples to wells in duplicate or triplicate.
   Note: When collecting supernatant from the cultured cells, be careful not to include cell debris. The following steps are recommended to eliminate deviation. First, harvest cells by centrifuging. Second, perform a filtration using 0.22μm filter.
2. Induce pre-reaction by adding 50μl of N1 buffer to the samples from step 1. And incubate for 5-10min at room temperature.
3. Carry out final reaction by adding 50μl of N2 buffer.
4. Leave the solution for 10 minutes in room temperature, and measure the absorbance value between 520-560nm using a plate reader. You may now calculate the nitrite concentration based on standard curve formula.

PROTOCOL II (For standard curve)

1. Designate triplicates in the 96 well plate for the nitrite standard curve (Fig).
2. Dispense 100μl of ultra pure water into wells in line 2-10 (or rows B-H).
3. Add 200μl of 1mM Nitrite Standard solution to the wells in line 1 (or row A).
4. Perform 10 serial 2-fold dilutions (Fig. 1) or 6 serial 2-fold dilutions (Fig. 2) in triplicate to generate the Nitrite Standard reference curve (1000, 500, 250 , 125, 62.5, 31.25, 15.63, 7.81, 3.91, 1.95, 0.98μM or 1000, 500, 250, 125, 62.5, 31.25, 15.63μM), discarding 100μl from the 0.98μM or 15.63μM set of wells, respectively. Do not add any nitrite solution to the last set of wells, which is negative control (0μM). The final volume in each well is 100μl, and the nitrite concentration range is 0 - 1,000μM (1mM).
5. Induce pre-reaction by adding 50μl of N1 buffer to the samples from step 1. And incubate for 5-10min at room temperature.
6. Carry out final reaction by adding 50μl of N2 buffer.
7. Leave the solution for 10 minutes in room temperature, and measure the absorbance value between 520-560nm using a plate reader.
8. According to above absorbance, you may generate a Nitrite Standard reference curve. Plot the average absorbance value of each concentration of the Nitrite Standard as a function of "Y" with nitrite concentration as a function of "X".

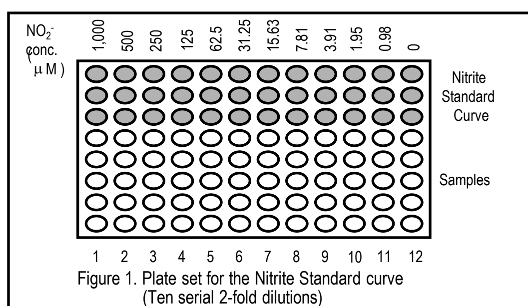

Figure 1. Plate set for the Nitrite Standard curve (Ten serial 2-fold dilutions)

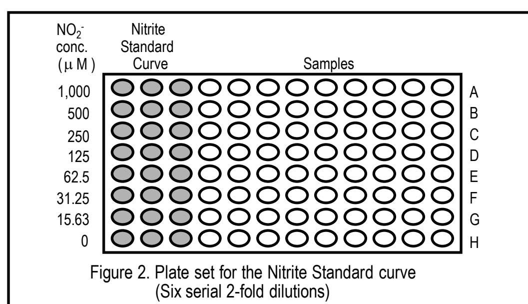

Figure 2. Plate set for the Nitrite Standard curve (Six serial 2-fold dilutions)

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