KAMIYA BIOMEDICAL COMPANY

Nitric Oxide Fluorometric Assay Kit

For the measurement of total nitric oxide (NO) in culture media, plasma, serum, urine and other biological fluids.

Cat. No. KT-152

For research use only, not for use in diagnostic procedures.

PRODUCT INFORMATION

Nitric Oxide Fluorometric Assay Kit
Cat. No. KT-152
200 tests

PRODUCT
The **K-ASSAY**® Nitric Oxide Fluorometric Assay Kit is a convenient fluorometric measurement kit for total nitrtic oxide (NO) in culture media, plasma, serum, urine and other biological fluids.

PRINCIPLE
Nitric oxide (NO) plays an important role in neurotransmission, vascular regulation, immune responses and apoptosis. Since most of the NO is oxidized to nitrite (NO_2^-) and nitrate (NO_3^-), the concentrations of these anions have been used as a quantitative measure of NO production. The **K-ASSAY**® Nitric Oxide Fluorometric Assay Kit provides an accurate and convenient measurement of total nitrate/nitrite concentration in a simple two-step process. The first step is the conversion of nitrate to nitrite utilizing nitrate reductase. The second step involves addition of DAN followed by NaOH, which converts nitrite into a fluorescent compound. Measurement of the fluorescence of this compound accurately determines the total nitric oxide production. The kit has been validated in culture media, plasma, and tissue homogenates. Some sample purification from these sources may be necessary.

COMPONENT

• 50X Assay Buffer	1 vial	2 ml
• Enzyme Co-factors	2 vials	Lyophilized
• Nitrate Reductase	2 vials	Lyophilized
• Nitrate Standard	1 vial	Lyophilized
• DAN Reagent	1 vial	5 ml
• Sodium Hydroxide	1 vial	5 ml
• Microtiter Plate	2 plates	
• Plate Cover	2 covers	

PROTOCOLS

A. Preparation of reagents

1. **Assay Buffer**: Dilute the 50X Assay Buffer to 100 ml with ddH_20. The buffer should be used for dilution of samples as needed prior to assay. Store at 4°C.
2. **Enzyme Cofactors**: Reconstitute with 1.2 ml of Assay Buffer. Keep on ice during use. Store at –20°C when not in use. Freeze/thaw of the solution should be limited to 1 time.
3. **Nitrate Reductase**: Reconstitute to 1.2 ml of Assay Buffer. Keep on ice during use. Store at –20°C when not in use. Freeze/thaw of the solution should be limited to 1 time.
4. **Nitrate Standard**: Reconstitute with 1.0 ml of Assay Buffer. Vertex and mix well. Store at 4°C when not in use (do not freeze!). The reconstituted standard is stable for 4 months when stored at 4°C.
5. **Fluorometric Reagent DAN** and **NaOH**: Ready to use. Store at 4°C.

B. Measurement of Nitrate + Nitrite

1. Prepare nitrate standard: A nitrate standard curve must be performed in order to quantitate sample nitrate + nitrite concentrations. Mix 0.9-ml of Assay Buffer with 0.1-ml reconstituted Nitrate Standard and vertex. Use this diluted standard (200 μM) for the preparation of the nitrate standard curve as described below.

- Obtain eight clean test tubes and number them #1 through #8.
- Aliquot 950 µl of Assay Buffer to #1 and 500 µl of Assay Buffer to #2-8.
- Transfer 50 µl of Nitrate standard into #1 and mix thoroughly. The concentration of standard in tube #1 is 10 µM.
- Serially dilute the nitrate by removing 500 µl from tube #1 and placing in tube #2, mix thoroughly.
- Next remove 500 µl from tube #2 and place it into tube #3, mix thoroughly. Repeat the process for tube #4-8.
- Do not store these standard for more than 24 hours.

Tube #	Nitrate Conc. (µM)	Nitrate (pmol/well)
1	10	500
2	5	250
3	2.5	125
4	1.25	62.5
5	0.625	31.2
6	0.313	15.6
7	0.156	7.8
8	0.078	3.9

2. Prepare nitrate standard curve:

- Reserve 9 wells for each standard curve (If using a single-cell fluorometer, perform all reactions in a small test tube).
- Add 80 µl of Assay Buffer to the first standard well and 30 µl to each of the remaining 8 wells.
- Add 50 µl of the nitrate standard tube #8 to the second standard well on the plate.
- Add 50 µl of tube #7 to the next standard well. Continue with the procedure for standard tube #6-#1.

3. Aliquot Samples: Add 10-20 µl of sample to the wells and adjust the volume to 80 µl with Assay buffer (note: Plasma samples and tissue homogenates should be assayed with no more than 10 µl of undiluted sample per well).

4. Add 10 µl of the Enzyme Cofactor mixture to each well.

5. Add 10 µl of the Nitrate Reductase mixture to each well.

6. Cover the plate with the plate cover and incubate at room temperature (RT) for 0.5-2 hrs.

7. Add 10 µl of DAN Reagent to each well. Incubate for 10 min. at RT.

8. Add 20 µl of NaOH to each well.

9. Read the plate in a fluorometer using Ex. = 375 nm and Em. = 415 nm. Alternatively, excitation and emission wavelengths of 360-365 and 430-450 nm, respectively, can also be used.

Calculations

1. Plot standard curve: Plot fluorescence vs. picomoles nitrate.
2. Determine sample nitrate and nitrite concentrations:

$$[\text{Nitrate + Nitrite}]\ (\text{nM}) = \left(\frac{\text{fluorescence} - \text{y-intercept}}{\text{slope}}\right)\left(\frac{1}{\text{sample volume } (\mu\text{l})}\right) \times \text{dilution}^*$$

*Where dilution is the sample dilution done prior to addition of the sample to the plate (or tube).

STORAGE

Store at -20°C.

FOR RESEARCH USE ONLY

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