Amplite® Fluorimetric Nitrite Quantification Assay Kit *Blue Fluorescence*

Catalog number: 21667
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 21667)
Component A: Nitrite Blue	Freeze (< -15 °C), Minimize light exposure	1 vial (25 µL)
Component B: Nitrite Assay Buffer	Freeze (< -15 °C), Minimize light exposure	10 mL
Component C: Nitrite Developer	Freeze (< -15 °C), Minimize light exposure	1 vial (1.5 mL)
Component D: Nitrite Standard-50 mM	Freeze (< -15 °C), Minimize light exposure	1 vial (25 µL)

OVERVIEW

The Amplite® Fluorimetric Nitrite Quantification Assay Kit is a highly sensitive and reliable method for measuring nitrite levels in biological samples, including cell culture supernatants and serum. Nitrite is a crucial precursor in the nitrate-nitrite-nitric oxide (NO) pathway, which plays a significant role in vasodilation, immune responses, and cellular signaling. Accurate quantification of nitrite is essential for researchers studying NO metabolism and its implications in various physiological and pathological processes.

This assay utilizes a fluorescence-based detection method, wherein nitrite reacts with a developer reagent to produce a fluorescent signal. The fluorescence intensity is directly proportional to the nitrite concentration, allowing for precise quantification using a fluorescence microplate reader. The kit includes optimized reagents for efficient and reproducible results, featuring a simple protocol with minimal hands-on time. Its high compatibility with various biological samples makes it an excellent tool for researchers investigating nitric oxide production and its role in health and disease.

AT A GLANCE

Protocol summary

1. Prepare test samples along with serially diluted nitrite standards (50 µL).
2. Add Nitrite Blue working solution (50 µL).
3. Incubate at RT for 10 minutes.
4. Add 15 µL of Nitrite Developer.
5. Measure the Fluorescence at Ex/Em=365/450 nm, Cut-off=435 nm.

Important notes
Thaw all the kit components at room temperature before starting the experiment and centrifuge the vials briefly before opening.

KEY PARAMETERS

Instrument: Fluorescence microplate reader

Fluorescence: Ex/Em =365/450 nm, Cut-off=435 nm

Recommended plate: Black Solid 96 well plate

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/21667

Nitrite Standard Dilution:
Add 2 µL of 50 mM nitrite standard solution (Component D) into 1 mL of distilled water to get 100 µM nitrite standard solution (STD 7). Take 500 µL (STD 7) and perform 2X serial dilutions in distilled water to get serially diluted nitrite standards (STD 6 to STD 1).

PREPARATION OF WORKING SOLUTION

Add 20 µL of Nitrite Blue (Component A) to 5 mL of Nitrite Assay Buffer (Component B). Mix well, protected from light.

Note: This working solution is enough for one 96-well plate. It is unstable at room temperature, and should be used promptly within 2 hours and avoid exposure to light. Alternatively, one can prepare the needed working solution proportionally.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1: Layout of Nitrite standards and test samples in a clear bottom 96-well microplate. STD = Nitrite Standards (STD 1-STD 7, 1.55 to 100µM), BL= Blank Control, TS = Test Samples.

BL	BL	Positive Control	TS
STD 1	STD 1	...	...
STD 2	STD 2	...	...
STD 3	STD 3		...
STD 4	STD 4		
STD 5	STD 5		
STD 6	STD 6		
STD 7	STD 7		

Table 2: Reagent composition for each well.

Well	Volume	Reagent
STD1-STD7	50 µL	Serial Dilutions (1.55 to 100 µM)
BL	50 µL	dd H2O
TS	50 µL	Test Sample

1. Prepare nitrite standards (STD 1-7), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 uL of reagent per well instead of 50 uL.
2. Add 50 uL of Nitrite Blue Working Solution to each well of blank control, standard and test samples. For a 384-well plate, add 25 uL of nitrite Working Solution into each well instead.
3. Incubate at RT for 10 minutes, protected from light.
4. Add 15 µL Nitrite Developer (Component C) to each well of blank control, standard and test samples. For a 384-well plate, add 7.5 uL of Nitrite Developer (Component C) into each well instead.
5. Incubate at RT for 5 min.
6. Measure fluorescence intensity with a fluorescence microplate reader at Ex/Em = 365/450 nm, Cut-off = 435 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The Fluorescence reading in blank wells is used as a control and is subtracted from the values of those wells with the nitrite standards, and test samples. The standard curve of nitrite is shown in Figure 1. To calculate the nitrite concentrations of the samples according to the standard curve, we recommend using the Online Linear Regression Calculator which can be found at: https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

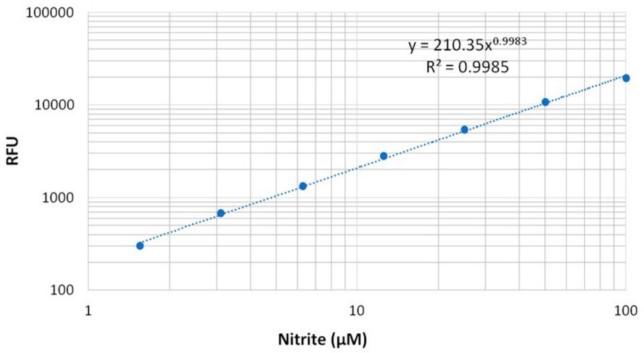

Figure 1. Nitrite dose responses were measured with the Nitrite Quantification assay kit on a 96-well clear bottom black solid microplate using a Gemini microplate reader (Molecular Devices) at Ex/Em = 365/450 nm, Cut-off = 435 nm.

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