Amplite™ Fluorimetric Extracellular Nitric Oxide (NO) Activity Assay Kit

Catalog number: 16365
Unit size: 100 Tests

Component	Storage	Amount
Component A: DAW-J2	Freeze (<-15 °C), Minimize light exposure	1 Vial
Component B: Assay Buffer	Freeze (<-15 °C)	1 Bottle (10 mL)
Component C: DMSO	Freeze (<-15 °C)	1 Vial (50 µL)

OVERVIEW

Nitric oxide (NO) is an important biological regulator involved in numbers of physiological and pathological processes. Altered NO production is implicated in various immunological, cardiovascular, neurodegenerative and inflammatory diseases. As a free radical, NO is rapidly oxidized and there is relatively low concentrations of NO existing in biological systems. It has been challenging to detect and understand the role of NO using conventional tools. AAT Bioquest offers a group of NO assay kits for monitoring NO in live cells. This Amplite™ Extracellular Fluorimetric Nitric Oxide Quantitation Kit provides a rapid method to monitor NO level in extracellular media, tissues, and other biological solutions and samples. Compared to the commonly used DAF-2 probe, DAW-J2 is used as the cell-impermeant NO sensor in our kit 16365. DAW-J2 detects nitric oxide exclusively in the extracellular environment with high sensitivity and selectivity. The non-fluorescent DAW-J2 probe reacts with NO to generate a strongly red fluorescent product that can be conveniently monitored using a microplate reader.

AT A GLANCE

Protocol summary

1. Prepare nitric oxide working solution (50 µL)
2. Add nitric oxide standard or test samples (50 µL)
3. Incubate at room temperature for 30 - 60 minutes
4. Monitor the fluorescence intensity

Important Thaw one of each kit component at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	570 nm
Emission:	610 nm
Cutoff:	590 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. *DAW-J2 stock solution (200X):*
   Add 25 mL of DMSO into the vial of DAW-J2 (Component A) to make 200X stock solution.

2. *Nitric oxide standard solution (100 mM, not provided):*
   We used DEA NONOate (Cayman Chemical, Item No. 82100, CAS#372965-00-9) as the nitric oxide standard. The standard solution of nitric oxide was prepared at the concentration of 100 mM in 0.01N NaOH.

PREPARATION OF STANDARD SOLUTION

Nitric Oxide standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/16365

Add 10 µL of 100 mM NONOate standard solution into 990 uL Assay Buffer (Component B) to generate 1 mM Nitric Oxide standard solution (SD7). Then perform 1:2 serial dilutions to get serially diluted nitric oxide standards (SD6-SD1).

Note Diluted NONOate standard solution is unstable, and should be used promptly.

PREPARATION OF WORKING SOLUTION

Add 25 µL of DAW-J2 200X stock solution into 5 mL of Assay Buffer (Component B) and mix well.

Note This nitric oxide working solution is enough for 100 assays. The working solution is not stable, use it promptly and avoid direct exposure to light.

SAMPLE EXPERIMENTAL PROTOCOL

Table1: Layout of nitric oxide standards and test samples in a solid black 96-well microplate. SD=Standard, BL=Blank Control, TS=Test Sample.

BL	BL	TS	TS
SD1	SD1	….	….
SD2	SD2		
SD3	SD3		
SD4	SD4		
SD5	SD5		
SD6	SD6		
SD7	SD7		

Table2: Reagent composition for each well.

Well	Volume	Reagent
SD1-SD7	50 µL	Serial Dilution (15.6 to 1000 µM)
BL	50 µL	Assay Buffer
TS	50 µL	Test Sample

1. Prepare nitric oxide standards (SD), blank controls (BL) and test samples (TS) into a solid black 96-well microplate according to the layout provided in Table 1 and Table 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL per well.

2. Add 50 µL of nitric oxide working solution to each well of standards, blank

controls and test samples to make the total nitric oxide assay volume of 100 µL/well. For a 384-well plate, use 25 µL of working solution into each well instead, for a total volume of 50 µL/well.

3. Incubate the reaction at room temperature for 30 minutes to 1 hour, protected from light.

4. Monitor the fluorescence increase with a fluorescence plate reader at Excitation = 570 nm, Emission = 610 nm (cutoff = 590 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU (Ex/Em=570/610 nm)) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Nitric Oxide Concentration samples. 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

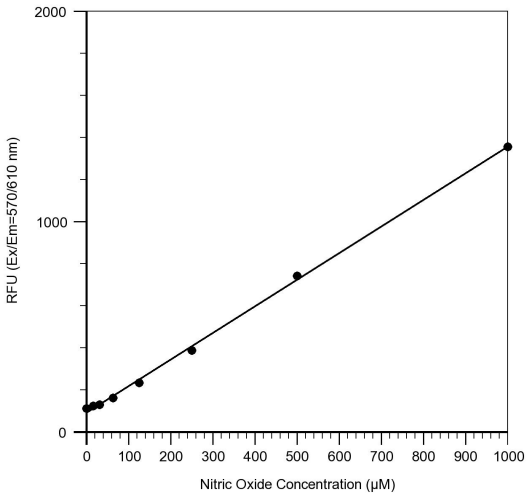

Figure 1. Nitric oxide dose response was measured with Amplite™ Extracelluar Fluorimetric Nitric Oxide Quantitation Kit in a 96-well solid black plate using a Gemini microplate reader (Molecular Devices).

DISCLAIMER