Amplite™ Fluorimetric Peroxynitrite Quantification Kit *Green Fluorescence*

Catalog number: 16316
Unit size: 100 Tests

Component	Storage	Amount
Component A: DAX-J2™ PON Green 99	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (20 mL)
Component C: DMSO	Freeze (<-15 °C)	1 vial (100 µL)

OVERVIEW

Peroxynitrite (ONOO-) is a strong oxidizing species and a highly active nitrating agent. Peroxynitrite is formed from the reaction between superoxide radicals and nitric oxide generated in cells. It can damage a wide array of biomolecules including proteins, enzymes, lipids and nucleic acids, eventually contributing to cell death. Due to its extremely short half-life and low steady-state concentration, it has been challenging to detect and quantify peroxynitrite in solution. In order to address this need, AAT Bioquest's Amplite™ Fluorimetric Peroxynitrite Quantification Kit provides a sensitive tool to measure ONOO- in solution. DAX-J2™ PON Green 99 reacts with ONOO- to generate a bright green fluorescent product. It specifically reacts with ONOO- with high selectivity over other reactive oxygen species (ROS) and reactive nitrogen species (RNS). This kit can be used with a fluorescence microplate reader and spectrometer.

AT A GLANCE

Important Thaw all the kit components to room temperature before use.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	490 nm
Emission:	530 nm
Cutoff:	515 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. DAX-J2™ PON Green 99 stock solution (500X):
Add 20 µL of DMSO (Component C) into the vial of DAX-J2™ PON Green 99 (Component A) to make 500X stock solution.

Note 20 µL of reconstituted DAX-J2™ PON Green 99 stock solution is enough for 1 plate. Keep from light.

2. Peroxynitrite ($ONOO^-$) stock solution (not provided):
Peroxynitrite stock solution was synthesized according to literature report. Briefly, a mixture of sodium nitrite (0.6 M) and hydrogen peroxide (0.7 M) was acidified with hydrochloric acid (0.6 M), and sodium hydroxide (1.5 M) was added within 1 - 2 seconds to make the solution alkaline. The excess hydrogen peroxide was removed by passing the solution through a short column of manganese dioxide. The extinction coefficient of peroxynitrite solution in 0.1 M NaOH is 1670 $M^{-1}cm^{-1}$ at 302 nm. The $ONOO^-$ stock solution is not stable; we highly recommend make it fresh to use.

PREPARATION OF STANDARD SOLUTION

Peroxynitrite standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/16316

Dilute Peroxynitrite ($ONOO^-$) stock solution in Assay buffer (Component B) to have 20 µM $ONOO^-$ standard solution, and then perform 1:2 serial dilutions to get serially diluted $ONOO^-$ standard solution (O7 - O1).

PREPARATION OF WORKING SOLUTION

Add 20 µL of 500X DAX-J2™ PON Green 99 stock solution into 10 mL of Assay Buffer (Component B) and mix well.

Note This assay mixture is enough for one 96-well plate. Protect from light.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of $ONOO^-$ standards and test samples in a solid black 96-well microplate. O = $ONOO^-$ Standards (O1 - O7, 0.313 to 20 µM); BL = Blank Control; TS = Test Samples.

BL	BL	TS	TS
O1	O1	...	...
O2	O2	...	...
O3	O3		
O4	O4		
O5	O5		
O6	O6		
O7	O7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
O1 - O7	50 µL	Serial Dilutions (0.313 to 20 µM)
BL	50 µL	assay buffer
TS	50 µL	test sample

1. Prepare $ONOO^-$ standards (O), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.
2. Add 50 µL of working solution to each well of $ONOO^-$ standard, blank control, and test samples to make the total $ONOO^-$ assay volume of 100 µL/well. For a 384-well plate, add 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 5 to 10 minutes, protected from light.
4. Monitor the fluorescence increase at Ex/Em = 490/530 nm (cutoff at 515 nm) with a fluorescence plate reader.

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EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) 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 Peroxynitrite samples. We recommend using the Online Four Parameter Logistics Calculator which can be found at:

https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator

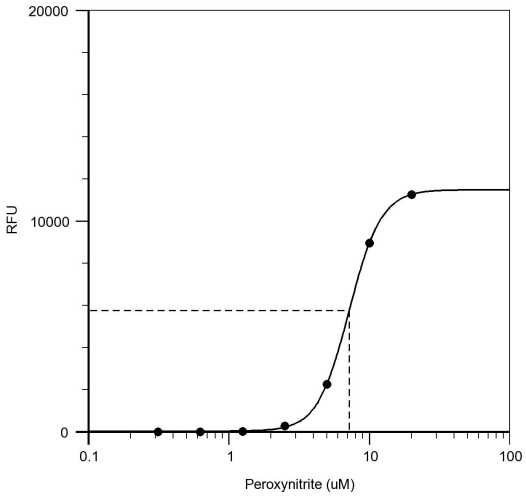

Figure 1. Peroxynitrite was measured with Amplite™ Fluorimetric Peroxynitrite Quantification Kit on a solid black 96-well plate using a Gemini microplate reader (Molecular Devices).

DISCLAIMER

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