Cell Meter™ Fluorimetric Intracellular Peroxynitrite Assay Kit *Green Fluorescence*

Catalog number: 16315
Unit size: 100 Tests

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

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 cause damages to a wide array of biomolecules including proteins, enzymes, lipids and nucleic acids, eventually contributing to cell death. Meanwhile, peroxynitrite can also have protective activities in vivo by contributing to host-defense responses against invading pathogens. Therefore, peroxynitrite is an essential biological oxidant involved in a board range of physiological and pathological processes. Due to its extremely short half-life and low steady-state concentration, it has been challenging to detect and understand the role of peroxynitrite in biological systems. AAT Bioquest's DAX-J2™ PON Green has been developed to address this umet need. It provides a sensitive tool to monitor ONOO- level in living cells. AAT Bioquest's DAX-J2™ PON Green specifically reacts with intercellular ONOO- to generate a bright green fluorescent product. It can be used in fluorescence imaging, flow cytometry and fluorescence microplate reader-based assays.

AT A GLANCE

Protocol summary

1. Prepare cells in growth medium
2. Co-incubate cells with test compounds and DAX-J2™ PON Green working solution at 37°C for desired period of time
3. Monitor fluorescence intensity at Ex/Em = 490/530 nm (Cutoff=515 nm)

Important Bring all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	490 nm
Emission:	530 nm
Cutoff:	515 nm
Instrument specification(s):	Bottom read mode
Recommended plate:	Black wall/clear bottom

Instrument:	Fluorescence microscope
Excitation:	490 nm
Emission:	530 nm
Instrument specification(s):	FITC Filter Set
Recommended plate:	Black wall/clear bottom

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 stock solution (500X):
Add 20 µL of DMSO (Component C) into the vial of DAX-J2™ PON Green (Component A), and mix well.

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

PREPARATION OF WORKING SOLUTION

Add 10 µL of 500X DMSO reconstituted DAX-J2™ Peroxynitrite Sensor stock solution into 500 µL of Assay Buffer (Component B) and mix well.

Note The working solution is not stable; prepare it as needed before use.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

1. Add 10 µL/well (96-well plate), or 2.5 µL/well (384-well plate) of DAX-J2™ PON Green working solution in 90 µL (96-well plate) or 22.5 µL (384-well plate) cell culture per well in the cell plate.

   Note It is not necessary to wash cells before staining. It's recommended to stain the cells in full medium.

2. Co-incubate cells with DAX-J2™ PON Green with test compounds in full medium or in your desired buffer at 37°C for desired period of time, protected from light. For control wells (untreated cells), add the corresponding amount of compound buffer.

   Note It's recommended to stain the cells in full medium. However, if tested compounds are serum sensitive, growth medium and serum factors can be aspirated away before staining. Add 90 µL/well (96-well plate) and 22.5 µL/well (384-well plate) of 1X Hank's salt solution and 20 mM Hepes buffer (HHBS) or the buffer of your choice after aspiration. Alternatively, cells can be stained in serum-free media. We co-incubated RAW 264.7 macrophage cells with 50 - 200 µM SIN-1 and DAX-J2™ PON Green in full medium at 37°C for 1 hour to induce peroxynitrite. See Figure 1 for details.

3. Alternatively, stain cells with DAX-J2™ PON Green at 37°C for 1 hour, protected from light. Remove the working solution, then treat cells with test compounds in full medium or in your desired buffer at 37°C for desired period of time.

4. Monitor the fluorescence increase using microplate reader at Ex/Em = 490/530 nm (cut off = 515 nm) with bottom read mode, or take images using fluorescence microscope with a FITC filter.

EXAMPLE DATA ANALYSIS AND FIGURES

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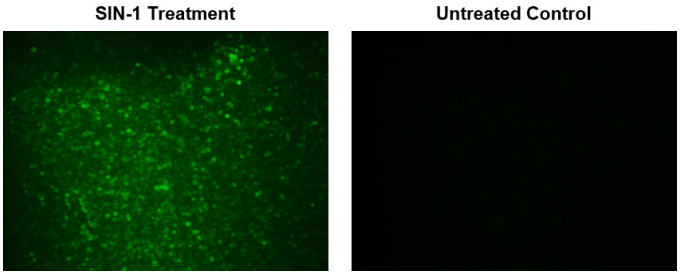

Figure 1. Fluorescence images of intracellular peroxynitrite in RAW 264.7 macrophage cells using Cell Meter™ Fluorimetric Intracellular Peroxynitrite Assay Kit (Cat#16315). Raw 264.7 cells at 100,000 cells/well/100 µL were seeded overnight in a Costar black wall/clear bottom 96-well plate. SIN-1 Treatment: Cells were co-incubated with DAX-J2™ PON Green and 100 µM SIN-1 at 37 °C for 1 hour. Untreated control: The RAW 264.7 cells were incubated with DAX-J2™ PON Green without SIN-1 treatment. The fluorescence signals were measured using a fluorescence microscope with a FITC filter

DISCLAIMER