Cell Meter™ Fluorimetric Intracellular Peroxynitrite Assay Kit *Optimized for Flow Cytometry*

Catalog number: 16317
Unit size: 100 Tests

Component	Storage	Amount
Component A: DAX-J2™ PON Green	Freeze (< -15 °C), Minimize light exposure	2 vials
Component B: 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 damage 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 broad 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. In order to address this unmet need, AAT Bioquest's Cell Meter™ Fluorimetric Intracellular Peroxynitrite (ONOO-) Assay Kit provides a sensitive tool to monitor ONOO- level in living cells. AAT Bioquest's DAX-J2™ PON Green is developed as an excellent fluorescent probe, which can specifically react with intercellular ONOO' to generate a bright green fluorescent product. This kit is optimized for flow cytometry.

AT A GLANCE

Protocol Summary

1. Prepare cells in growth medium
2. Co-incubate cells with test compounds (to stimulate $ONOO^-$) and DAX-J2™ PON Green working solution
3. Monitor fluorescence intensity at Ex/Em = 490/530 nm

Important Allow all the components to warm to room temperature before begining protocol.

KEY PARAMETERS

Flow cytometer

Excitation	488 nm laser
Emission	530/30 nm filter
Instrument specification(s)	FITC channel

CELL PREPARATION

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

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.

DAX-J2™ PON Green stock solution (400X)
Add 25 µL of DMSO (Component C) into the vial of DAX-J2™ PON Green (Component A), and mix well. **Note:** 1 µL of reconstituted DAX-J2™ PON Green stock solution is enough for 0.4 mL of cells.

SAMPLE EXPERIMENTAL PROTOCOL

1. 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 is 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. Resuspend cells in 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 Jurkat cells with 200 µM SIN-1 and DAX-J2™ PON Green in full medium at 37°C for 1 hour to induce peroxynitrite. See Figure 1(A) for details.

2. 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. See Figure 1(B) for details.

3. Monitor the fluorescence intensity at FITC channel (Ex/Em=490/530 nm) using a flow cytometer. Gate on the cells of interest, excluding debris.

EXAMPLE DATA ANALYSIS AND FIGURES

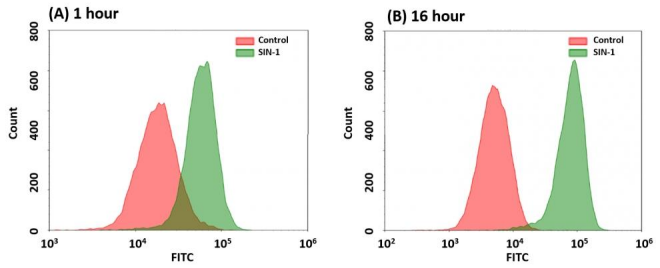

Figure 1.
Detection of peroxynitrite in Jurkat cells upon SIN-1 treatment using AAT Cell Meter™ Fluorimetric Intracellular Peroxynitrite Assay Kit (Cat#16317). (A) Jurkat cells were co-incubated with DAX-J2™ PON Green and 200 µM SIN-1 in full medium at 37 ºC for 1 hour. (B) Cells were stained with DAX-J2™ PON Green for 1 hour, washed with PBS and then incubated with 200 µM SIN-1 in full medium at 37 ºC for 16 hours. Cells stained with DAX-J2™ PON Green but without SIN-1 treatment were used as a control. Fluorescence intensity was measured using ACEA NovoCyte flow cytometer in FITC channel.

DISCLAIMER

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