Cell Meter™ Fluorimetric Intracellular Peroxynitrite Assay Kit

Green Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 16315 (100 assays)	Keep in freezer Protect from light	Fluorescence microplate readers, Fluorescence Microscope

Introduction

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 Cell Meter™ Fluorimetric Intracellular Peroxynitrite ($ONOO^-$) Assay Kit 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 is developed as an excellent fluorescent probe, which can specifically react with intercellular $ONOO^-$ to generate a bright green fluorescent product. This kit can be used in fluorescence imaging and fluorescence microplate reader.

Kit Components

Components	**Amount**
Component A: DAX-J2™ PON Green	1 vial
Component B: Assay Buffer	1 vial (1 mL/vial)
Component C: DMSO	1 vial (100 µL/vial)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare cells in growth medium → Co-incubate cells with test compounds and DAX-J2™ PON Green working solution → Monitor fluorescence intensity at Ex/Em = 490/530 nm

1. Prepare cells:

1.1. For adherent cells: Plate cells overnight in growth medium at 20,000 to 80,000 cells/well/90 µL for a 96-well plate or 5,000 to 20,000 cells/well/22.5 µL for a 384-well plate.

1.2. For non-adherent cells: Centrifuge the cells from the culture medium and suspend the cell pellets in culture medium at 80,000-200,000 cells/well/90 µL for a 96-well poly-D lysine plate or 20,000-50,000 cells/well/22.5 µL for a 384-well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to your experiment.
Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density.

2. Prepare working solution:

2.1 Prepare 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 them well.
Note: 20 µL of reconstituted DAX-J2™ PON Green stock solution is enough for 1 plate. Unused portion can be aliquoted and stored at ≤ -20 °C for more than one month if the tubes are sealed tightly and kept from light. Avoid repeated freeze-thaw cycles.

2.2 Prepare DAX-J2™ PON Green working solution (10X): Add 10 µL of 500X DMSO reconstituted DAX-J2™ Peroxynitrite Sensor stock solution (from Step 2.1) into 500 µL of Assay Buffer (Component B), and mix them well.
Note: The working solution is not stable; prepare it as needed before use.

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3. Run the peroxynitrite assay:

3.1 Add 10 µL/well (96-well plate), or 2.5 µL/well (384-well plate) of DAX-J2™ PON Green working solution (from Step 2.2) in 90 µL (96-well plate) or 22.5 µL (384-well plate) cell culture per well in the cell plate (from Step 1).
Note: It is not necessary to wash cells before staining. It's recommended to stain the cells in full medium.

3.2 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 1: 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 90µL/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.
Note 2: We co-incubated RAW 264.7 macrophage cells with 50-200 µM SIN-1 and DAX-J2™ PON Green in full medium at 37ºC for 1 hour to induce peroxynitrite. See Figure 1 for details.

3.3 Alternatively, stain cells with DAX-J2™ PON Green at 37ºC for 1 hour, protected from light (as in Step 3.1). Remove the working solution, then treat cells with test compounds in full medium or in your desired buffer at 37ºC for desired period of time.

3.4 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.

Data Analysis

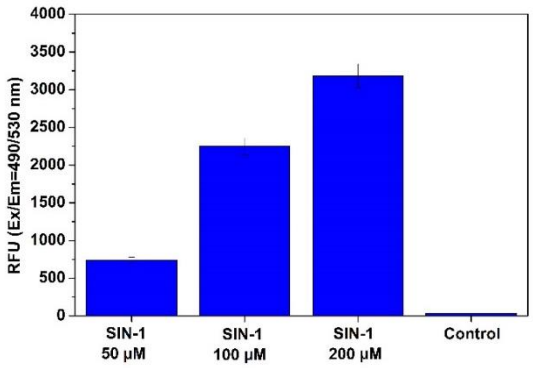

Figure 1. Detection of peroxynitrite in living cells upon SIN-1 treatment using Cell Meter™ Fluorimetric Intracellular Peroxynitrite Assay Kit (Cat#16315). RAW 264.7 cells at 100,000 cells/well/100 µL were seeded overnight in a Costar black wall/clear bottom 96-well plate. Cells were co-incubated with DAX-J2™ PON Green working solution and SIN-1 at the concentration from 50 to 200 µM at 37 ºC for 1 hour. Cells incubated with DAX-J2™ PON Green without SIN-1 treatment were used as control. The fluorescence signal were monitored at Ex/Em = 490/530 nm (cut off = 515 nm) with bottom read mode using a FlexStation microplate reader (Molecular Devices).

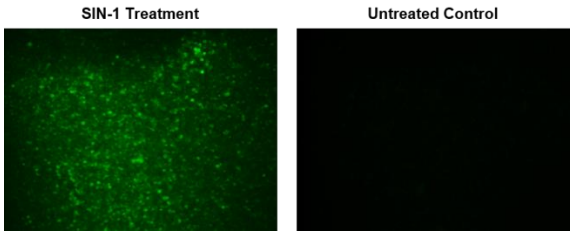

Figure 2. Fluorescence images of intracellular peroxynitrite in RAW 264.7 macrophage cells using Cell Meter™ Fluorimetric Intracellular Peroxynitrite Assay Kit (Cat#16315). Raw 264.7 cells at 100,000 cells/well/100 µL were seeded overnight in a Costar black wall/clear bottom 96-well plate. SIN-1 Treatment: Cells were co-incubated with DAX-J2™ PON Green and 100 µM SIN-1 at 37 ºC 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.

References

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