

Version 1 Last updated 29 March 2018

ab233468

Peroxynitrite Assay Kit

(Cell-based)

For the measurement of peroxynitrite in adherent and non-adherent cells

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Due to its extremely short half-life and low steady-state concentration, it has been challenging to detect and understand the role of peroxynitrite (ONOO^-) in biological systems. Peroxynitrite Assay Kit (Cell-based) (ab233468) has been developed to address this unmet need. It provides a sensitive tool to monitor ONOO^- level in living cells. Peroxynitrite Sensor 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.

Prepare cells.



Prepare Peroxynitrite Sensor Green (500X) stock solution and Peroxynitrite Sensor Green (10X) working solution.



Co-incubate cells with Peroxynitrite Sensor Green working solution and test compounds at 37°C in the dark for the desired period of time.

OR

Pre-incubate cells with Peroxynitrite Sensor Green working solution for 1 hour at 37°C in the dark. Remove Peroxynitrite Sensor Green and add test compounds for the desired period of time.



Monitor fluorescence intensity at Ex/Em = 490/530 nm.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Reconstituted components are stable for more than 1 month.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)
Peroxynitrite Sensor Green	1 vial	-20°C
Assay Buffer	1 mL	-20°C
DMSO	100 µL	-20°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of reading fluorescence at Ex/Em = 490/530 nm or fluorescent microscope with FITC filter.
- 37°C cell incubator.
- 96- or 384-well plates.
- Cell culture medium / buffer.
- Test compound of interest.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:

www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Peroxynitrite Sensor Green stock solution (500X)

Add 20 μL of DMSO into the vial of Peroxynitrite Sensor Green and mix well to make 500X stock solution.

Δ Note 20 μL of Peroxynitrite Sensor Green (500X) is enough for 1 plate.

Δ Note Unused Peroxynitrite Sensor Green (500X) can be aliquoted and stored at -20°C in tightly sealed tubes. Avoid light and freeze-thaw cycles.

5.2 Peroxynitrite Sensor Green working solution (10X)

Add 10 μL of 500X DMSO reconstituted Peroxynitrite Sensor Green (from step 5.1) into 500 μL of Assay Buffer and mix well.

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

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6. Sample Preparation

6.1 Adherent cells:

Plate cells overnight in growth medium at 20,000 to 80,000 cells/well/90 μ L for a 96-well plate or 5,000 to 20,000 cells/well/22.5 μ L for a 384-well plate.

6.2 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 μ L for a 96-well poly-D lysine plate or 20,000-50,000 cells/well/22.5 μ L for a 384-well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to your experiment.

Δ Note Each cell line should be evaluated on an individual basis to determine the optimal cell density.

7. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

7.1 Run the peroxynitrite assay:

EITHER

1. Add 10 μL /well (96-well plate), or 2.5 μL /well (384-well plate) of Peroxynitrite Sensor Green working solution (from Step 5.2) in 90 μL (96-well plate) or 22.5 μL (384-well plate) cell culture per well in the cell plate.

Δ 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 Peroxynitrite Sensor Green (10X) and test compounds in your growth medium / buffer of choice at 37°C for the desired period of time, protected from light.

Δ Note It is recommended to stain the cells in full medium. However, if the test 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 or the buffer of your choice after aspiration. Alternatively, cells can be stained in serum-free media.

Δ Note: By way of example, we co-incubated RAW 264.7 macrophage cells with 50-200 μM SIN-1 Peroxynitrite Sensor Green in full medium at 37°C for 1 hour to induce peroxynitrite.

OR

3. Pre-stain cells Peroxynitrite Sensor Green at 37°C for 1 hour in your growth medium / buffer of choice protected from light (as in 7.1.1) but in the absence of test compounds.
4. Remove the cell medium, then treat the cells with test compounds in the growth medium / buffer of your choice (without Peroxynitrite Sensor Green) at 37°C for the desired period of time.

BOTH

5. Measure the fluorescence using a microplate reader at Ex/Em=490/530 nm or a fluorescence microscope using the FITC filter.

8. FAQs / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

9. Typical Data

Data provided for demonstration purposes only.

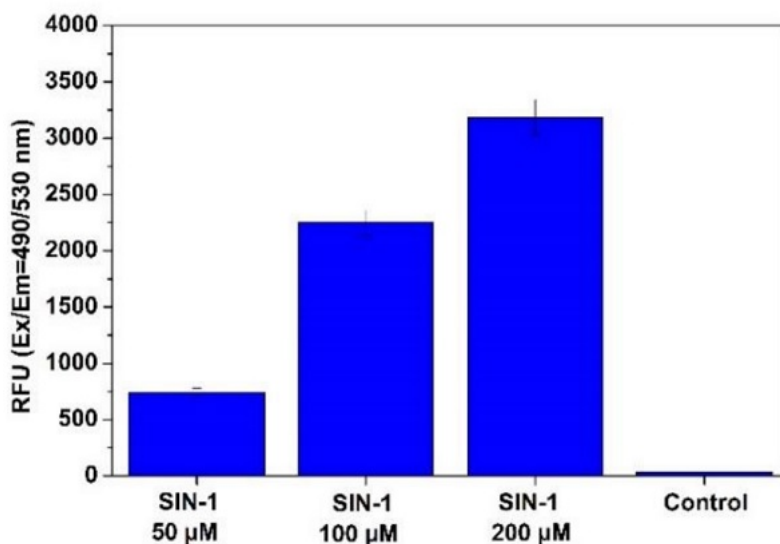


Figure 1. Detection of peroxynitrite in living cells upon SIN-1 treatment using ab233468 Peroxynitrite Assay Kit (Cell-based). RAW 264.7 cells at 100,000 cells/well/100 μ L were seeded overnight in a Costar black wall/clear bottom 96-well plate. Cells were co-incubated with Peroxynitrite Sensor Green working solution and SIN-1 at the concentration from 50 to 200 μ M at 37 $^{\circ}$ C for 1 hour. Cells incubated with Peroxynitrite Sensor 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.

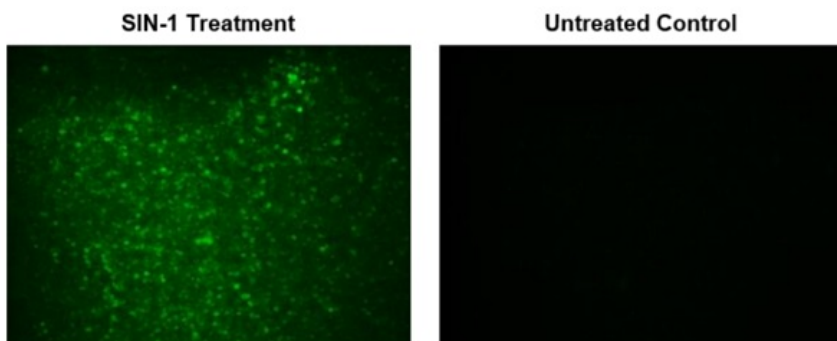


Figure 2. Fluorescence images of intracellular peroxynitrite in RAW 264.7 macrophage cells using ab233468 Peroxynitrite Assay Kit (Cell-based). RAW 264.7 cells at 100,000 cells/well/100 μ L were seeded overnight in a Costar black wall/clear bottom 96-well plate. SIN-1 Treatment: Cells were co-incubated with Peroxynitrite Sensor Green working solution and 100 μ M SIN-1 at 37°C for 1 hour. Untreated control: The RAW 264.7 cells were incubated with Peroxynitrite Sensor Green without SIN-1 treatment. The fluorescence signals were measured using a fluorescence microscope with a FITC filter.

10. Notes

Technical Support

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Austria

wissenschaftlicherdienst@abcam.com | 019-288-259

France

supportscientifique@abcam.com | 01.46.94.62.96

Germany

wissenschaftlicherdienst@abcam.com | 030-896-779-154

Spain

soportecientifico@abcam.com | 91-114-65-60

Switzerland

technical@abcam.com

Deutsch: 043-501-64-24 | Français: 061-500-05-30

UK, EU and ROW

technical@abcam.com | +44(0)1223-696000

Canada

ca.technical@abcam.com | 877-749-8807

US and Latin America

us.technical@abcam.com | 888-772-2226

Asia Pacific

hk.technical@abcam.com | (852) 2603-6823

China

cn.technical@abcam.com | 400 921 0189 / +86 21 2070 0500

Japan

technical@abcam.co.jp | +81-(0)3-6231-0940

Singapore

sg.technical@abcam.com | 800 188-5244

Australia

au.technical@abcam.com | +61-(0)3-8652-1450

New Zealand

nz.technical@abc.com | +64-(0)9-909-7829