

Version 1 Last updated 28 March 2018

ab233470 Peroxynitrite Assay Kit (Cell-based, Flow cytometry)

For the measurement of peroxynitrite in living 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 in biological systems. In order to address this unmet need, ab233470 Peroxynitrite Assay Kit (Cell-based, Flow cytometry) provides a sensitive tool to monitor ONOO⁻ levels 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 is optimized for flow cytometry.

Prepare cells.



Prepare Peroxynitrite Sensor Green (400X) stock solution.



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

OR

Pre-incubate cells with Peroxynitrite Sensor Green 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 at FITC channel (Ex/Em = 490/530 nm) in a flow cytometer.

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)	Storage temperature (after prep)
Peroxynitrite Sensor Green	2 vials	-20°C	-20°C
DMSO	100 µL	-20°C	N/A

3. Materials Required, Not Supplied

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

- Flow cytometer with FITC filter (Ex/Em = 490/530 nm).
- 37°C cell incubator.
- Cell culture 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 (400X)

Add 25 μL of DMSO into the vial of Peroxynitrite Sensor Green and mix well to make 400X stock solution.

Δ Note: 1 μL of Peroxynitrite Sensor Green (400X) is for 0.4 mL of cells.

Δ Note: Unused Peroxynitrite Sensor Green (400X) can be aliquoted and stored at -20°C in tightly sealed tubes, avoid light and freeze-thaw cycles.

6. Sample Preparation

General sample information:

Prepare cells in 0.5 mL growth medium or buffer of your choice at a density of 5×10^5 – 1×10^6 cells/mL.

ΔNote: Each cell line should be evaluated individually to determine the optimal cell density for peroxynitrite induction.

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 Prepare cells:

Prepare cells as described above (Sample Preparation, Section 6).

7.2 Prepare Peroxynitrite Sensor Green (400X):

Prepare Peroxynitrite Sensor Green (400X) as described above (Reagent Preparation, Section 5.1).

7.3 Incubate cells with Peroxynitrite Sensor Green:

EITHER

1. Co-incubate cells with Peroxynitrite Sensor Green (400X) (1 μ L/0.4 mL of cells) 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 μ M SIN-1 Peroxynitrite Sensor Green in full medium at 37°C for 1 hour to induce peroxynitrite.

OR

2. Pre-stain cells Peroxynitrite Sensor Green at 37°C for 1 hour in your growth medium / buffer of choice protected from light (as in 7.3. 1., above) but in the absence of test compounds.
3. 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

4. At the end of the incubation period (co-incubation or pre-stain protocol), monitor the fluorescence intensity at the FITC channel (Ex/Em=490/530 nm) using a flow cytometer. Gate on the cells of interest, excluding debris.

8. FAQs / Troubleshooting

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

9. Typical Data

Data provided for demonstration purposes only.

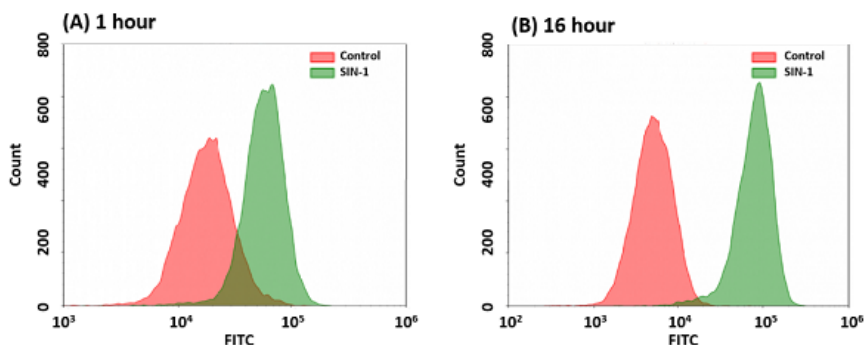


Figure 1. Detection of peroxynitrite in Jurkat cells following SIN-1 treatment using ab233470 Peroxynitrite Assay Kit (Cell-based, Flow cytometry).

(A) Jurkat cells were co-incubated with Peroxynitrite Sensor Green and 200 μ M SIN-1 in full medium at 37 $^{\circ}$ C for 1 hour.

(B) Cells were pre-stained with Peroxynitrite Sensor Green for 1 hour, washed with PBS and then incubated with 200 μ M SIN-1 in full medium at 37 $^{\circ}$ C for 16 hours.

Cells stained with Peroxynitrite Sensor Green but without SIN-1 treatment were used as a control. Fluorescence intensity was measured using an ACEA NovoCyte flow cytometer in the FITC channel.

10. Notes

Technical Support

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