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ab233469 Peroxynitrite Assay Kit (Fluorometric)

For the measurement of peroxynitrite in solution.

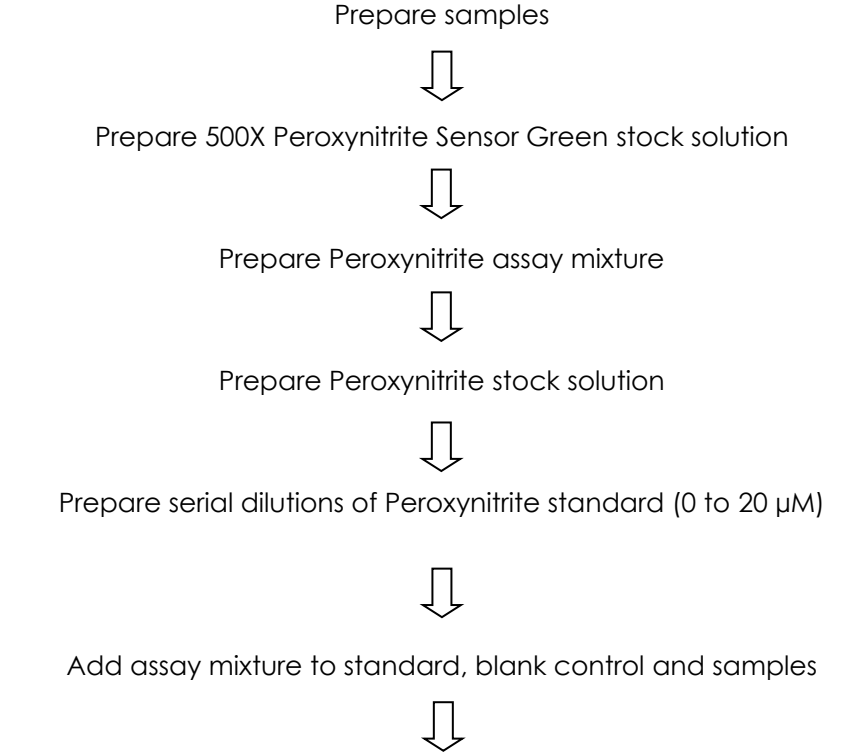
This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Peroxynitrite Assay Kit (Fluorometric) (ab233469) provides a sensitive tool to measure peroxynitrite in solution.

Peroxynitrite Sensor Green reacts with $ONOO^-$ to generate a bright green fluorescent product. It specifically reacts with $ONOO^-$ with high selectivity over other reactive oxygen species (ROS) and reactive nitrogen species (RNS). This kit can be used with a fluorescence microplate reader and spectrometer.



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.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Peroxynitrite Sensor Green	1 vial	-20 °C	-20°C
Assay Buffer	1 bottle (20 mL)	-20 °C	-20°C
DMSO	1 vial (100 µL)	-20 °C	-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 measuring fluorescence at Ex/Em = 490/530 nm
- 96 well plate with clear flat bottom, preferably black (for fluorometric assay)
- Peroxynitrite stock solution

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

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

5.2 Assay buffer

Ready to use as supplied.

5.3 DMSO

Ready to use as supplied.

5.4 Peroxynitrite stock solution (not provided)

Peroxynitrite stock solution was synthesized according to literature report. A mixture of sodium nitrite (0.6 M) and hydrogen peroxide (0.7 M) was acidified with hydrochloric acid (0.6 M), and sodium hydroxide (1.5 M) was added within 1-2 seconds to make the solution alkaline. The excess hydrogen peroxide was removed by passing the solution through a short column of manganese dioxide. The extinction coefficient of peroxynitrite solution in 0.1 M NaOH is 1670 M-1cm-1 at 302 nm.

Δ Note: *The peroxynitrite stock solution is not stable; you might store it at ≤ -80 °C for 2-4 weeks, we highly recommend make it fresh to use.*

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. Dilute the Peroxynitrite stock solution (described in step 5.4) in assay buffer to make a 20 µM Peroxynitrite standard solution.

2. Perform 1:2 serial dilutions to get approximately 20, 10, 5, 2.5, 1.25, 0.625, 0.313 and 0 µM.

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 500X Peroxynitrite Sensor Green stock solution:

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

Δ Note: *20 µL of reconstituted Peroxynitrite Sensor 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.*

7.2 Prepare Peroxynitrite assay mixture:

Add 20 µL of 500X Peroxynitrite Sensor Green stock solution (from Step 7.1) into 10 mL of Assay Buffer, and mix them well.

Δ Note: *This assay mixture is enough for one 96-well plate. It is stable at 4°C for 2 hours when protected from light.*

7.3 Prepare $ONOO^-$ stock solution (not provided):

See preparation notes in section 5.4

7.4 Prepare serial dilutions of $ONOO^-$ standard (0 to 20 µM):

1. Preparation shown in section 6.
2. Add peroxynitrite standards and peroxynitrite containing test samples into a 96-well solid black microplate, as shown in table 1 and 2.

7.5 Run Peroxynitrite assay:

1. Add 50 µL of assay mixture (from Step 7.2) to each well of the $ONOO^-$ standard, blank control, and test samples to make the total assay volume of 100 µL/well.
   Δ Note: *For a 384-well plate, add 25 µL sample and 25 µL of assay mixture per well.*
2. Incubate the reaction at room temperature for 5 to 10 minutes, protected from light.
3. Monitor the fluorescence increase at Ex/Em = 490/530 nm (cutoff at 515 nm) with a fluorescence plate reader.

BL	BL	TS	TS	…	…						
O1	O1	…	…	…	…						
O2	O2										
O3	O3										
O4	O4										
O5	O5										
O6	O6										
O7	O7										

Δ Note: *O= $ONOO^-$ Standards; BL= Blank Control; TS= Test Samples*

Table 1: Layout of Peroxynitrite standards and test samples in a solid black 96-well microplate.

$ONNOO^-$ Standard	Blank Control	Test Sample
Serial dilutions (50 µL)	TE: 50 µL	50 µL

Δ Note: *Add the serial dilutions of $ONNOO^-$ standards from 0.313 to 20 µM into wells from O1 to O7 in duplicate.*

Table 2: Reagent composition for each well.

8. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with the Peroxynitrite reactions. A Peroxynitrite standard curve is shown in Figure 1. Calculate the Peroxynitrite concentration of the samples according to the Peroxynitrite standard curve.

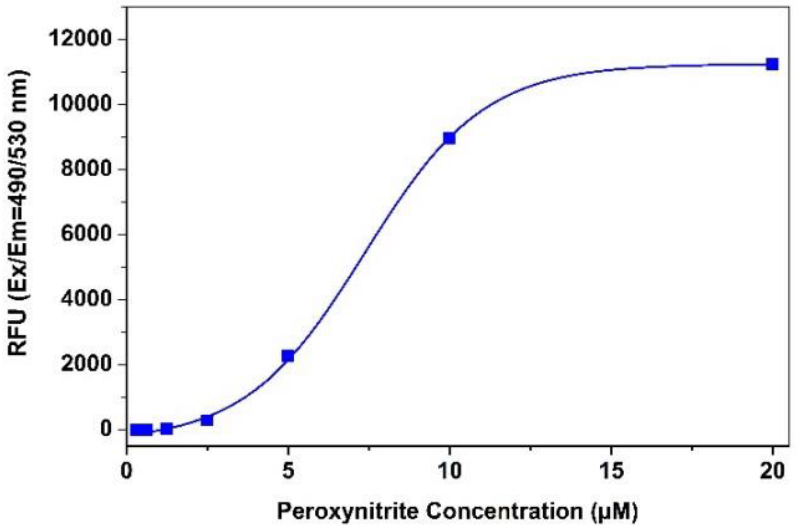


Figure 1: Peroxynitrite was measured with the Peroxynitrite Quantification Kit (Fluorometric) (ab233469) on a solid black 96-well plate using a Gemini microplate reader. As low as 1.25 µM was detected. (**Δ Note:** *The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.*)

9. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)