ab138874

Hydrogen Peroxide Assay Kit (Cell-based)

Instructions for Use

This kit provides a sensitive, one-step fluorometric assay to quantify hydrogen peroxide in live cells.

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

Version 3a, Last updated November 6, 2023

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1. Introduction

Hydrogen peroxide (H_2O_2) is a reactive oxygen metabolic by-product that serves as a key regulator for a number of oxidative stress-related states. It is involved in many biological events that are linked to asthma, atherosclerosis, diabetic vasculopathy, osteoporosis, a number of neurodegenerative diseases and Down's syndrome. The measurement of this reactive species is helpful for determining how oxidative stress modulates various intracellular pathways.

ab138874 Hydrogen Peroxide Assay Kit (Cell-based) uses our unique AbGreen Indicator to quantify hydrogen peroxide in live cells. AbGreen Indicator is cell-permeable, and generates the green fluorescence when it reacts with hydrogen peroxide. The kit is an optimized "mix and read" assay format that is compatible with HTS liquid handling instruments. ab138874 provides a sensitive, one-step fluorometric assay to detect as little as 0.3 nanomoles of H_2O_2 in a 100 µL assay volume (3 µM). The assay can be performed in a convenient 96-well or 384-well microtiter-plate format. Its signal can be easily read by either a fluorescence microplate reader at Ex/Em = 490/520 nm (bottom read mode) for H_2O_2 detection in solution or a fluorescence microscopy for live cell H_2O_2 imaging.

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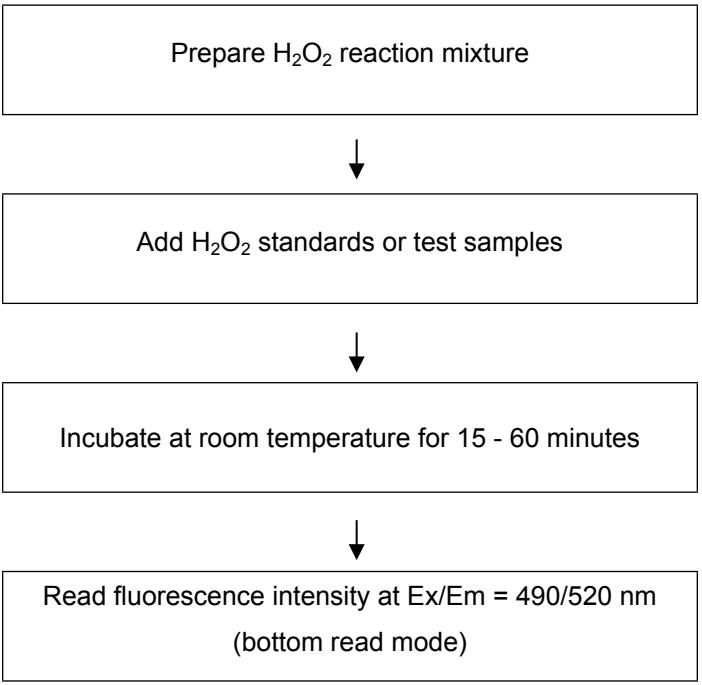
Kit Key Features

- **Broad Application:** Can be used for quantifying hydrogen peroxide in live cells, in solutions, and in cell extracts.
- **Continuous:** Easily adapted to automation without a separation step.
- **Convenient:** Formulated to have minimal hands-on time.
- **Non-Radioactive:** No special requirements for waste treatment

2. Protocol Summary

Summary for One 96-well Plate



Note: Thaw all the kit components to room temperature before starting the experiment.

3. Kit Contents

Components	Amount
Component A: AbGreen Indicator	1 vial
Component B: Hydrogen Peroxide Solution	1 vial (3% stabilized solution, 200 µL)
Component C: Assay Buffer	1 bottle (20 mL)
Component D: DMSO	1 vial (200 µL)

4. Storage and Handling

Keep at -20°C. Avoid exposure to light.

5. Additional Materials Required

- 96 or 384-well microplates: Black microplates with clear bottoms.
- Fluorescent microplate reader
- PBS Buffer

6. Assay Protocol

Note: This protocol is for one 96 - well plate.

A. Prepare Stock Solutions

1. AbGreen Indicator stock solution (250X): Add 50 µL of DMSO (Component D) into the vial of AbGreen Indicator (Component A). The stock solution should be used promptly. Any remaining solution should be aliquoted and refrozen at -20 °C.

   Note: Avoid repeated freeze-thaw cycles and protect from light.

2. 20 mM H_2O_2 stock solution: Add 22.7 µL of 3% H_2O_2 (0.88 M, Component B) into 977µL of Assay Buffer (Component C).

   Note: The diluted H_2O_2 solution is not stable. The unused portion should be discarded.

B. Prepare AbGreen Indicator working solution

Add 20 µL of AbGreen Indicator stock solution (250X) into 5 mL of Assay Buffer (Component C).

C. Prepare serial dilutions of H_2O_2 standards (0 to 1000 µM):

1. Add 50 µL of 20 mM H_2O_2 solution into 950 µL of Assay Buffer (Component C) to get 1000 µM H_2O_2 solution.

2. Take 200 µL of 1000 µM H_2O_2 solution to perform 1:3 serial dilutions to get 300, 100, 30, 10, 3, 1, 0.3 and 0 µM serially diluted H_2O_2 standards.

3. Add H_2O_2 standards and H_2O_2-containing test samples into a black 96-well microplate with a clear bottom as described in Tables 1 and 2.

BL	BL	TS	TS	….	….						
HS1	HS1	….	….	….	….						
HS2	HS2										
HS3	HS3										
HS4	HS4										
HS5	HS5										
HS6	HS6										
HS7	HS7										

Table 1. Layout of H_2O_2 standards and test samples in a black 96-well microplate with a clear bottom.

Note: HS= H_2O_2 *Standards; BL=Blank Control; TS=Test Sample*

H_2O_2 Standards	Blank Control	Test Sample
Serial Dilutions: 50 µL	Assay Buffer: 50 µL	50 µL

Table 2. Reagent composition for each well.

D. Run H_2O_2 assay in supernatants reaction:

1. Add 50 µL of 1X AbGreen Indicator working solution to each well of the H_2O_2 standard, blank control, and test samples to make the total H_2O_2 assay volume of 100 µL/well.
   Note: For a 384-well plate, add 25 µL of sample and 25 µL of acetylthiocholine reaction mixture in each well.

2. Incubate the reaction for 15 to 60 minutes at room temperature, protected from light.

3. Monitor the fluorescence increase at Ex/Em = 490±10/520±10 nm (optimal Ex/Em = 490/520) with a fluorescence plate reader (bottom read mode).

E. Run H_2O_2 assay in live cells:

AbGreen Indicator can be loaded passively into living cells and report the micromolar changes in intracellular H_2O_2 concentrations. The following is a suggested microscope imaging protocol that can be modified to meet specific research needs

1. Activate the cells as desired.

2. Wash the cells with PBS buffer, incubate the cells with 100 µL/well 1X AbGreen Indicator working solution for 5 to 60 minutes or your desired time.
   Note: For a 384-well plate, add 25 µL/well of 1X AbGreen Indicator working solution. We recommend optimising concentration and incubation time of AbGreen Indicator for the used cell type.

3. Monitor the fluorescence increase at excitation 490 nm and emission at 525nm using a fluorescence plate reader with bottom read mode. Or image the fluorescence change with a fluorescence microscope using FITC channel.

7. Data Analysis

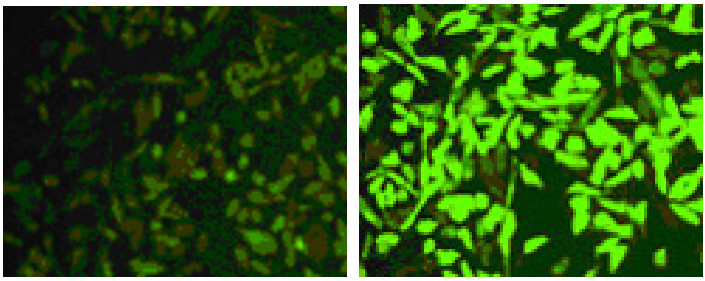


Figure 1. Images of Live CHO-K1 cells in a black 96-well plate. Live CHO-K1 cells were stained with ab138874.

Left Image: Control cells.

Right Image: Cells treated with 100 µM H_2O_2 at room temperature for 5 minutes.

8. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

Technical Support

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