Amplite™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit

Green Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 11503 (200 assays)	Keep in freezer Avoid exposure to light	Fluorescence microplate readers

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.

This Amplite™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit uses our unique ROS Green™ hydrogen peroxide sensor to quantify hydrogen peroxide in live cells. ROS Green™ 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. The Amplite™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit 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 for H_2O_2 detection in solution or a fluorescence microscopy for live cell H_2O_2 imaging.

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. No wash is required.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Components	**Amount**
Component A: Amplite™ Green Peroxide Sensor	1 vial
Component B: H_2O_2	1 vial (3% stabilized solution, 200 µL)
Component C: Assay Buffer	1 bottle (20 mL)
Component D: DMSO	1 vial (200 µL)

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Assay Protocol for One 96-well Plate

Brief Summary

Prepare H_2O_2 reaction mixture (50 µL) → Add H_2O_2 standards or test samples (50 µL)→ Incubate at room temperature for 15-60 minutes → Read fluorescence intensity at Ex/Em = 490/520 nm

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

1. Prepare stock solutions:

1.1 Amplite™ Green Peroxide Sensor stock solution (250X): Add 50 µL of DMSO (Component D) into the vial of Amplite™ Green Peroxide Sensor (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.

1.2 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.

2. Prepare 1X Amplite™ Green Peroxide Sensor working solution:

Add 20 µL of Amplite™ Green Peroxide Sensor stock solution (250X, from Step 1.1) into 5 mL of Assay Buffer (Component C).

3. Prepare serially diluted H_2O_2 standards (0 to 1000 µM):

3.1 Add 50 µL of 20 mM H_2O_2 solution (from Step 1.2) into 950 µL of Assay Buffer (Component C) to get 1000 µM H_2O_2 solution.

3.2 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 stands.

3.3 Add H_2O_2 standards and H_2O_2-containing test samples into a solid black 96-well microplate as described in Tables 1 and 2.

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

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

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

Table 2. Reagent composition for each well

H_2O_2 Standards	**Blank Control**	**Test Sample**
Serial Dilutions*: 50 µL	Assay Buffer (Component C): 50 µL	50 µL

4. Run H_2O_2 assay in supernatants reaction:

4.1 Add 50 µL of 1X Amplite™ Green Peroxide Sensor working solution (from Step 2) to each well of the H_2O_2 standard, blank control, and test samples (see Step 3.3) 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 1X Amplite™ Green peroxide Sensor working solution into each well.

4.2 Incubate the reaction at room temperature for 15 to 30 minutes, protected from light.

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

5. Run H_2O_2 assay in live cells:

Amplite™ Green Peroxide Sensor 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.

5.1 Activate the cells as desired.

5.2 Wash the cells with PBS buffer, incubated the cells with 100 µL/well 1X Amplite™ Green Peroxide Sensor working solution (from Step 2) for 5 to 60 minutes or your desired time.
Note: For a 384-well plate, add 25 µL/well of 1X Amplite™ Green Peroxide Sensor working solution.

5.3 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.

Data Analysis

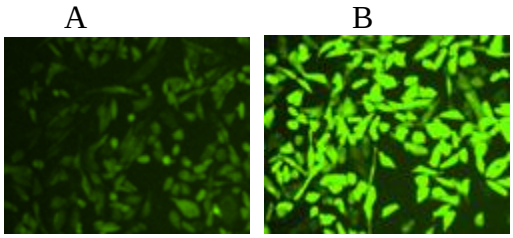

Figure 1. Images of Live CHO-K1 cells in a Costar black 96-well plate. Live CHO-K1 cells were stained with Amplite™ Intracellular Fluorimetric Hydrogen Peroxide Assay Kit. A: Control cells. B: Cells treated with 100 µM H_2O_2 at room temperature for 5 minutes.

References

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