

Amplite® IR

Catalog number: 11009
Unit size: 1 mg

Component	Storage	Amount (Cat No. 11009)
Amplite™ IR	Freeze (< -15 °C), Minimize light exposure, Desiccated	1 vial (1 mg)

OVERVIEW

Our Amplite® IR is a fluorogenic peroxidase substrate that generates near infrared fluorescence upon reaction with peroxidase and H₂O₂. It can be used to detect both H₂O₂ and peroxidase. Amplite® IR generates a substance that has maximum absorption of 647 nm with maximum emission at 670 nm. This near infrared absorption and fluorescence minimize the assay background that is often caused by the autoabsorption and/or autofluorescence of biological samples that rarely absorb light beyond 600 nm. Unlike other HRP substrates such as dihydrofluoresceins and dihydrorhodamines, the air-oxidation of Amplite® IR is minimal. Compared to Amplex Red™, Amplite® IR generates the fluorescence that is pH-independent from pH 4 to 10. In addition, it has excellent water solubility. It is a superior alternative to Amplex Red™ for the detections that require low pH where Amplex Red™ has significantly reduced fluorescence. We have used Amplite® IR to detect HRP in quite a few immunoassays. Amplite® IR can also be used to detect trace amount of H₂O₂. Because H₂O₂ is produced in many enzymatic redox reactions, Amplite® IR can be used in coupled enzymatic reactions to detect the activity of many oxidases and/or related enzymes/substrates or cofactors such as glucose, acetylcholine and cholesterol, L-glutamate, amino acids etc.

AT A GLANCE

Protocol Summary

1. Prepare 100 µM Amplite® IR with 0.8 U/mL peroxidase in phosphate buffer and add 50 µL in a well
2. Add H₂O₂ standards or test samples (50 µL)
3. Incubate at RT for 0-30 minutes
4. Monitor fluorescence intensity at Ex/Em = 640/680 nm

Important Note

The following is the recommended protocol for H₂O₂ assay in solution and live cells. The protocol only provides a guideline, should be modified according to the specific needs.

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

Amplite® IR stock solution

Add an appropriate amount of anhydrous DMSO to make 10 to 25 mM Amplite® IR stock solution.

PREPARATION OF WORKING SOLUTION

Amplite® IR working solution (2X)

To achieve a final concentration per well of 50 to 100 µM in 50 mM

phosphate buffer or buffer of your choice, make a 100 to 200 µM concentration solution in a tube. 50 µL is required per well.

Note: Amplite® IR is unstable in the presence of thiols such as DTT and b-mercaptoethanol. Thiols higher than 10 µM (final concentration) could significantly decrease the assay dynamic range. NADH and glutathione (reduced from GSH) may interfere with the assay.

Note: We recommend using a fresh stock solution every time you perform experiments.

SAMPLE EXPERIMENTAL PROTOCOL

Run H₂O₂ assay in supernatants

1. Add 50 µL of 2X Amplite® IR working solution (from Step 1.2) into each well of the H₂O₂ standard, blank control, and test samples to make the total H₂O₂ assay volume of 100 µL/well.

Note: For a 384-well plate, add 25 µL of sample and 25 µL of 2X Amplite® IR working solution into each well.

2. Incubate the reaction at room temperature for 0 to 30 minutes, protected from light.
 3. Monitor the fluorescence increase at Ex/Em = 640/680 nm with a fluorescence plate reader.
- Note:** Amplite® IR peroxidase substrate can readily self-oxidize, so read the fluorescence as soon as the H₂O₂ reaction mixture is added to increase the signal-to-noise ratio.
4. 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 H₂O₂

Run H₂O₂ assay for cells:

1. Amplite® IR can be used to measure the release of H₂O₂ from cells. The following is a suggested protocol that can be modified for your specific research needs. The Amplite® IR working solution should be prepared as Step 1.2 except that the phosphate buffer should be replaced with the media that is used in the cell culture system. Suggested media including (a) Krebs Ringers Phosphate Buffer (KRPB); (b). Hanks Balanced Salt Solution (HBSS); or (c) Serum-free media.
2. Prepare cells in a 96-well plate (50-100 µL/well), and activate the cells as desired.

Note: The negative controls (media alone and non-activated cells) are included for measuring background fluorescence.

3. Add 50 μL of H_2O_2 reaction mixture to each well of the cells, and those of H_2O_2 .

Note: For a 384-well plate, add 25 μL of cells and 25 μL of H_2O_2 reaction mixture into each well.

4. Incubate the reaction for 0 to 30 minutes at room temperature, protected from light.

5. Monitor the fluorescence increase at Ex/Em = 640/ 680 nm with a fluorescence plate reader.

Note: The contents of the plate can also be transferred to a white clear bottom plate and read by an absorbance microplate reader at the wavelength of 670 nm. The absorption detection has lower sensitivity compared to fluorescence reading.

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.

EXAMPLE DATA ANALYSIS AND FIGURES

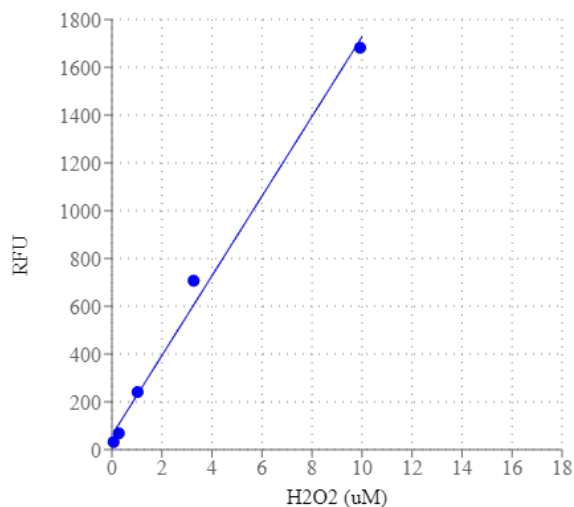


Figure 1. H₂O₂ dose response was measured in a solid black 96-well plate with Amplite® Fluorimetric Hydrogen Peroxide Assay Kit.

DISCLAIMER

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