

Amplite™ IR

Ordering Information:

Product Number: 11009 (1 mg)

Storage Conditions:

Keep at -20 °C and desiccated.
Expiration date is 12 months from the date of receipt.

Introduction

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 product that has maximum absorption of 647 nm with maximum emission at 670 nm. This near infrared absorption and fluorescence minimize the assay background which 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 ADHP (also called Amplex™ Red in literature), Amplite™ IR generates the fluorescence that is pH-independent ranging from pH 4 to 10. Thus it is a superior alternative to ADHP for the detections that require low pH where ADHP has 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 might be used in coupled enzymatic reactions to detect the activity of oxidases and/or related enzymes/substrates or cofactors such as glucose, acetylcholine, cholesterol, L-glutamate, and amino acids etc. Its signal can be readily read with either fluorescence microplate reader at Ex/Em = 640/680 nm or absorbance microplate reader at 650 nm.

Chemical and Physical Properties

Molecular Weight: ~400
Solvent: dimethylsulfoxide (DMSO)
Spectral Properties: Ex/Em = 647/670 nm

Assay Protocol with Amplite™ IR

Brief Summary

Prepare 100 μM Amplite™ IR with 0.8 U/mL peroxidase in phosphate buffer (50 μL) → Add H₂O₂ standards or test samples (50 μL) → Incubate at RT for 0-30 minutes → Read fluorescence intensity at Ex/Em = 640/680 nm

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.

1. Prepare Amplite™ IR working solution:

- 1.1 Prepare a 10 to 25 mM stock solution of Amplite™ IR in high-quality, anhydrous DMSO. The stock solution should be used promptly. Any unused solution need to be aliquoted and refrozen at ≤ -20 °C.

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

- 1.2 Prepare a 2X Amplite™ IR working solution: On the day of the experiment, either dissolve Amplite™ IR solid in DMSO or thaw an aliquot of the Amplite™ IR stock solution at room temperature. Prepare a 2X working solution of 100 to 250 μM in 50 mM phosphate buffer or buffer of your choice, pH 7 with 0.8 units/mL peroxidase. Amplite™ IR final concentration of 50 to 100 μM is recommended for measuring H₂O₂ concentration in solution.

Note: Amplite™ IR is unstable in the presence of thiols such as DTT and β-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.

2. Run H₂O₂ assay in supernatants:

- 2.1 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.

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

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

Note: Amplite™ IR peroxidase substrate is easy to be self-oxidized, so read the fluorescence as soon as the H₂O₂ reaction mixture is added to increase the signal to noise ratio.

- 2.4 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 reactions.

3. Run H₂O₂ assay for cells:

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.

- 3.1 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.

- 3.2 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.

- 3.3 Add 50 μL of H₂O₂ reaction mixture (from Step 1.2) to each well of the cells, and those of H₂O₂ standards.

Note: For a 384-well plate, add 25 μL of cells and 25 μL of H₂O₂ reaction mixture into each well.

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

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

Note1: 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.

Note2: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

Disclaimer: This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact our technical service representative for more information.