Amplite™ IR

Ordering Information:	*Storage Conditions:*
Product Number: #11009 (1 mg)	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 H_2O_2. It can be used to detect both H_2O_2 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 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 ADHP (also called Amplex Red™ in literature) Amplite™ IR generates the fluorescence that is pH-independent from pH 4 to 10. Thus it is 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 H_2O_2. Because H_2O_2 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 with Ex/Em = 640/680 nm or absorbance microplate reader at 650 nm.

Chemical and Physical Properties

Molecular Weight: ~400
Solvents: soluble in 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 H_2O_2 standards or test samples (50 µL) → Incubate at RT for 0-30 min → Read fluorescence at Ex/Em = 640/680 nm

Note: Following is our recommended protocol for H_2O_2 assay in solution and live cells. This protocol only provides a guideline, should be modified according to your 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 remaining solution need be aliquot and refrozen at $\leq$ -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 to 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 to100 µM is recommended for measuring H_2O_2 concentration in solution.

Note: Amplite™ IR is unstable in the presence of thiols such as DTT and β-mercaptoethanol. The final concentration of the thiols higher than 10 µM could significantly decreases the assay dynamic range. NADH and glutathione (reduced form: GSH) may interfere with the assay.

2. Run H_2O_2 assay in supernatants:

2.1 Add 50 µL of 2X Amplite™ IR working solution (from step 1.2) to each well of the H_2O_2 standard, blank control, and test samples so that the total H_2O_2 assay volume is 100 µL/well.
Note: For a 384-well plate, add 25 µL sample, 25 µL of 2X Amplite™ IR working solution per well.

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

2.3 Monitor the fluorescence increase with Ex/Em = 640/680 nm using a fluorescence plate reader.
Note: Amplite™ IR peroxidase substrate is easy to be self-oxidized, so read the fluorescence as soon as the H_2O_2 reaction mixture was 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 H_2O_2 reactions.

3. Run H_2O_2 assay for cells:

Amplite™ IR can be used to measure the release of H_2O_2 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 your 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 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 H_2O_2 reaction mixture (from step 1.2) to each well of the cells, and those of H_2O_2 standards.
Note: For a 384-well plate, add 25 µL cells, 25 µL of H_2O_2 reaction mixture per well.

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

3.5 Monitor the fluorescence increase with Ex/Em = 640/ 680 nm by using a fluorescence plate reader.
Note1: The contents of the plate can also be transferred to a white clear bottom plate and read by absorbance microplate reader at the wavelength 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.

www.interchim.com
interchim

211 bis Avenue Kennedy - BP 1140
03103 Montluçon - France
33 (0) 4 70 03 88 55
Fax 33 (0) 4 70 03 82 60
e-mail interchim@interchim.com

Agence Paris - Normandie
33 (0) 1 41 32 34 40
Fax 33 (0) 1 47 91 23 90
e-mail interchim.paris@interchim.com