Amplite™ Red HRP Substrate

Ordering Information:	*Storage Conditions:*
Product Number: 11011 (1,000 Assays)	Keep at -20 °C and desiccated

Introduction

Amplite™ Red HRP substrate is a sensitive fluorogenic peroxidase substrate that generates a highly red fluorescent product that has maximum absorption of 571 nm and maximum emission of 585 nm. Unlike other HRP substrates such as dihydrofluoresceins and dihydrorhodamines, the air-oxidation of Amplite™ Red is minimal. Amplite™ Red has been widely used to detect HRP in many immunoassays. Amplite™ Red can also be used to detect trace amount of H_2O_2. The Amplite™ Red based H_2O_2 detection is at least one order of magnitude more sensitive than the commonly used scopoletin assay for H_2O_2. Because H_2O_2 is produced in many enzymatic redox reactions, Amplite™ Red 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.

Spectral Properties

Ex/Em = 571/585 nm

Assay Protocol for Peroxidase (HRP) with Amplite™ Red (for one 96 well black plate)

Brief Summary

Prepare 1X Amplite™ Red working solution with 200 µM H_2O_2 in phosphate buffer (50 µL) → Add Peroxidase standards or test samples (50 µL) → Incubate at RT for 10-30 minutes → Monitor fluorescence intensity at Ex/Em = 540/590 nm

Note: The following is the recommended protocol for peroxidase assay in solution. The protocol only provides a guideline, should be modified according to the specific needs.

A1. Prepare Amplite™ Red peroxidase working solution:

1.1 Prepare 250X Amplite™ Red stock solution: Add 200 µL of anhydrous DMSO into the vial, mixed well. 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 1X Amplite™ Red peroxidase working solution: On the day of the experiment, either dissolve Amplite™ Red solid in DMSO or thaw an aliquot of the Amplite™ Red stock solution at room temperature. Prepare 1X working solution by adding 20 µL of 250X Amplite™ Red stock solution (from Step 1.1) in 5 mL of 50 mM phosphate buffer or buffer of your choice, pH 7 with 200 µM H_2O_2.
Note: Amplite™ Red 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.

A2. Run peroxidase assay in supernatants:

2.1 Add 50 µL of 1X Amplite™ Red peroxidase working solution (from Step 1.2) into each well of the peroxidase standard, blank control, and test samples to make the total peroxidase assay volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL of sample and 25 µL of 1X Amplite™ Red peroxidase working solution into each well.

2.2 Incubate the reaction at room temperature for 10 to 30 minutes, protected from light.

2.3 Monitor the fluorescence increase at Ex/Em = 540/590 nm with a fluorescence plate reader.

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 peroxidase reactions.

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Technical Support: support@aatbio.com; Tel; 408-733-1055

Assay Protocol for H_2O_2 with Amplite™ Red (for one 96 well black plate)

Brief Summary

Prepare 1X Amplite™ Red H_2O_2 working solution with 0.4 U/mL peroxidase in phosphate buffer (50 µL) → Add H_2O_2 standards or test samples (50 µL) → Incubate at RT for 10-30 minutes → Monitor fluorescence intensity at Ex/Em = 540/590 nm

Note: The following is the recommended protocol for H_2O_2 assay in solution. The protocol only provides a guideline, should be modified according to the specific needs.

B1. Prepare Amplite™ Red H_2O_2 working solution:

1.1 Prepare 250X Amplite™ Red stock solution: Add 200 µL of anhydrous DMSO into the vial, mixed well. 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 1X Amplite™ Red H_2O_2 working solution: On the day of the experiment, either dissolve Amplite™ Red solid in DMSO or thaw an aliquot of the Amplite™ Red stock solution at room temperature. Prepare 1X working solution by adding 20 µL of 250X stock solution (from Step 1) in 5 mL of 50 mM phosphate buffer or buffer of your choice, pH 7 with 0.4 units/mL peroxidase.
Note: Amplite™ Red 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.

B2. Run H_2O_2 assay in supernatants:

2.1 Add 50 µL of 1X Amplite™ Red H_2O_2 working solution (from Step 1.2) into 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 1X Amplite™ Red H_2O_2 working solution into each well.

2.2 Incubate the reaction at room temperature for 10 to 30 minutes, protected from light.

2.3 Monitor the fluorescence increase at Ex/Em = 540/590 nm with a fluorescence plate reader.

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