Amplite™ Red

Catalog number: 11011
Unit size: 1000 Assays

Component	Storage	Amount
Amplite™ Red HRP Substrate	Freeze (<-15 °C), Dessicated, Minimize light exposure	1,000 Assays

OVERVIEW

Our Amplite™ Red 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 is one of the most sensitive and stable fluorogenic probes for detecting HRP and H2O2. Amplite™ Red has been widely used to detect HRP in many immunoassays. On the other hand, Amplite™ Red can also be used to detect trace amount of H2O2. The Amplite™ Red-based H2O2 detection is at least one order of magnitude more sensitive than the commonly used scopoletin assay for H2O2. Because H2O2 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.

AT A GLANCE

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

1. Prepare and add 1X Amplite™ Red working solution with 200 mM H_2O_2 in phosphate buffer (50 µL)
2. Add Peroxidase standards or test samples (50 µL)
3. Incubate at room temperature for 10-30 minutes
4. Monitor fluorescence intensity at Ex/Em = 540/590 nm

Important 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.

Thaw one of each kit component at room temperature before starting the experiment.

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

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

Important 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.

Thaw one of each kit component at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	550 nm
Recommended plate:	Solid black

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™ Red stock solution (250X):
Add 200 mL 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 °.

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

PREPARATION OF WORKING SOLUTION

1. Amplite™ Red Peroxidase working solution (1X):
Add 20 µL of Amplite™ Red stock solution (250X) in 5 mL of 50 mM phosphate buffer or buffer of your choice, pH 7 with 200 mM H_2O_2.

Note Amplite™ Red 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.

2. Amplite™ Red H_2O_2 working solution (1X):
Add 20 µL of Amplite™ Red stock solution (250X) 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 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.

SAMPLE EXPERIMENTAL PROTOCOL

Peroxidase assay in supernatants

1. Add 50 µL of 1X Amplite™ Red peroxidase working solution 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. Incubate the reaction at room temperature for 10 to 30 minutes, protected from light.

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

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.

H_2O_2 assay in supernatants

1. Add 50 µL of 1X Amplite™ Red H_2O_2 working solution 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.

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

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

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.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate HRP samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

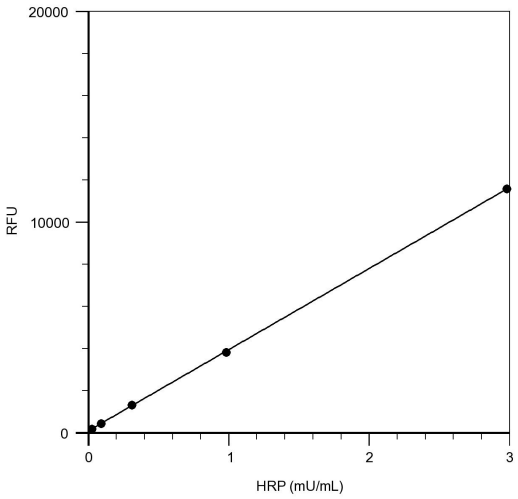

Figure 1. HRP dose response was measured with Amplite™ Fluorimetric Peroxidase Assay Kit in a black plate using a Gemini fluorescence microplate reader (Molecular Devices).

DISCLAIMER

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