Amplite® Red

Catalog number: 11011
Unit size: 1000 Assays

Component	Storage	Amount (Cat No. 11011)
Amplite® Red HRP Substrate	Freeze (< -15 °C), Minimize light exposure, Desiccated	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 µM 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 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.

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

Protocol Summary for H2O2 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 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.

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

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 µL of anhydrous DMSO (not provided) into the vial and mix well. The stock solution should be used promptly. Any unused solution needs to be aliquoted and refrozen at ≤-20°C.

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

PREPARATION OF WORKING SOLUTION

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 µM 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.

Amplite® Red H2O2 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.

H202 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.

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

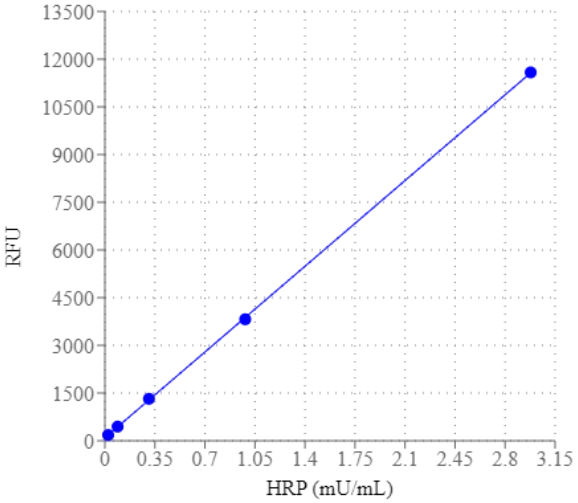

Figure 1. HRP dose response was measured with Amplite® Fluorimetric Peroxidase Assay Kit in a black plate using a Gemini fluorescence microplate reader (Molecular Devices).

DISCLAIMER

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