Amplite® Colorimetric Peroxidase (HRP) Assay Kit *Blue Color*

Catalog number: 11551
Unit size: 500 Tests

Component	Storage	Amount
Component A: Amplite™ Blue Peroxidase Substrate	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: H2O2	Freeze (< -15 °C), Minimize light exposure	1 vial (3% stabilized solution, 200 µL)
Component C: Assay Buffer	Freeze (< -15 °C)	1 bottle (100 mL)
Component D: Horseradish Peroxidase	Freeze (< -15 °C), Minimize light exposure	1 vial (20 units)
Component E: DMSO	Freeze (< -15 °C)	1 vial (0.5 mL)

OVERVIEW

Peroxidase is a small molecule (MW ~40 KD) that can usually be conjugated to an antibody in a 4:1 ratio. Due to its small size, it rarely causes steric hindrance problem with antibody/antigen complex formation. Peroxidase is inexpensive compared to other labeling enzymes. The major disadvantage associated with peroxidase is their low tolerance to many preservatives such as sodium azide that inactivates peroxidase activity even at low concentration. HRP conjugates are extensively used as secondary detection reagents in ELISAs, immuno-histochemical techniques and Northern, Southern and Western blot analyses. We offer this quick (10 min) HRP assay in a one-step, homogeneous, no wash assay system. This kit uses Amplite® Blue, our ultrasensitive chromogenic HRP substrate. Our Amplite® Blue is a chromogenic peroxidase substrate that is much more sensitive to both H2O2 and peroxidase than other chromogenic peroxidase substrates such as TMB, ABTS, OPD and K-Blue. Amplite® Blue generates a highly absorptive material that has maximum absorption of 664 nm. This near infrared absorption minimizes the background absorption that is often caused by the autoabsorption of biological samples that rarely absorb light beyond 600 nm. The kit can be used for ELISAs, characterizing kinetics of enzyme reaction and high throughput screening of oxidase inhibitors, etc. The kit provides an optimized 'mix and read' assay protocol that is compatible with HTS liquid handling instruments.

AT A GLANCE

Protocol Summary

1. Prepare HRP standards and/or test samples (50 µL)
2. Add HRP working solution (50 µL)
3. Incubate at room temperature for 10 - 30 minutes
4. Monitor absorbance at 664 ± 5 nm

Important Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Absorbance microplate reader

Absorbance	664 ± 5 nm
Recommended plate	Clear bottom

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.

1. Amplite™ Blue Peroxidase Substrate stock solution (100X)
Add 250 µL of DMSO (Component E) into the vial of Amplite™ Blue Substrate (Component A) to make 100X Amplite ™ Blue Peroxidase Substrate stock solution.

2. HRP standard solution (20 U/mL)
Add 1 mL of Assay Buffer (Component C) into the vial of HRP (Component D) to make 20 U/mL HRP stock solution.

3. H_2O_2 solution (20 mM)
Add 22.7 µL of 3% H_2O_2 (0.88 M, Component B) into 977 µL of Assay Buffer (Component C) to make 20 mM H_2O_2 solution. **Note:** The diluted H_2O_2 solution is not stable. The unused portion should be discarded.

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11551

HRP standard
Add 15 µL of 20 U/mL HRP solution into 985 µL of Assay Buffer (Component C) to get 300 mU/mL HRP standard solution (SD7). Take 300 mU/mL HRP standard solution (SD7) and perform 1:3 serial dilutions to get serially diluted HRP standards (SD6 - SD1) with Assay Buffer (Component C).

PREPARATION OF WORKING SOLUTION

Add 50 µL of Amplite™ Blue Peroxidase Substrate stock solution (100X) and 50 µL of H_2O_2 stock solution (20 mM) into 4.9 mL of Assay Buffer (Component C) to make a total volume of 5 mL HRP working solution. Protect from light.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of HRP standards and test samples in a white wall/clear bottom 96-well microplate. SD=HRP Standards (SD1 - SD7, 0.3 to 300 mU/mL); BL=Blank Control; TS=Test Samples.

BL	BL	TS	TS
SD1	SD1	...	...
SD2	SD2	...	...
SD3	SD3		
SD4	SD4		
SD5	SD5		
SD6	SD6		
SD7	SD7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
SD1 - SD7	50 µL	Serial Dilution (0.3 to 300 mU/mL)
BL	50 µL	Assay Buffer (Component C)
TS	50 µL	test sample

1. Prepare HRP standards (SD), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of HRP working solution to each well of HRP standard, blank control, and test samples to make the total HRP assay volume of 100 µL/well. For a 384-well plate, add 25 µL of HRP working solution into each well instead, for a total volume of 50 µL/well.

3. Incubate the reaction at room temperature for 30 to 60 minutes, protected from light.

4. Monitor the absorbance with an absorbance plate reader at 664 ± 5 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

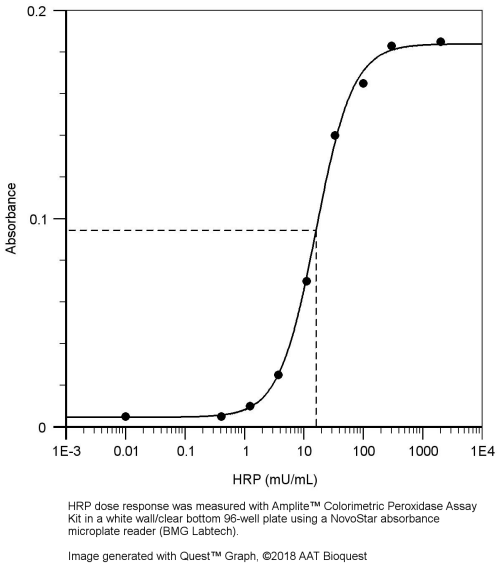

Figure 1. HRP dose response was measured with Amplite™ Colorimetric Peroxidase Assay Kit in a white wall/clear bottom 96-well plate using a NovoStar absorbance microplate reader (BMG Labtech).

DISCLAIMER

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