Amplite™ Luminometric Peroxidase Assay Kit

Ordering Information	Storage Conditions	Instrument Platform
Product Number: 11559 (500 assays)	Keep in freezer Avoid exposure to light	Luminescence microplate readers

Introduction

Enhanced chemiluminescence is a common technique for a variety of detection assays in biology. A horseradish peroxidase enzyme (HRP) is tethered to the molecule of interest (usually through labeling an immunoglobulin that specifically recognizes the molecule). This enzyme complex catalyzes the conversion of the enhanced chemiluminescent substrate into a sensitized reagent in the vicinity of the molecule of interest. The further oxidation of the substrate by hydrogen peroxide produces an excited molecule which emits light.

This kit uses our Amplite™ luminometric HRP substrate to quantify peroxidase in solutions. It provides an optimized "mix and read" assay protocol. Our Amplite™ Luminometric Peroxidase Assay Kit can detect as low as 100 µU/mL HRP. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format. Its signal can be easily read by a luminescence microplate reader. The kit can be used for ELISAs, characterizing kinetics of enzyme reaction and high throughput screenings.

Kit Key Features

Broad Application:	Can be used for quantifying HRP activities in solutions and solid surfaces (e.g, ELISA)
Sensitive:	Detect as low as 100 µU/mL HRP in solution.
Continuous:	Easily adapted to automation without a separation step.
Convenient:	Formulated to have minimal hands-on time. No wash is required.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Components	Amount
Component A: Assay Buffer	1 bottle (25 mL)
Component B: H_2O_2	1 vial (3% stabilized solution, 200 µL)
Component C: Horseradish Peroxidase	1 vial (20 units)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare HRP reaction mixture (50 µL) → Add HRP standards and/or test samples (50 µL) → Incubate at room temperature for 30 minutes to 2 hours → Monitor luminescent intensity

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

1. **Prepare HRP reaction mixture:**
   Add 30 µL of 3% stabilized H_2O_2 solution (Component B) into 5 mL of Assay Buffer (Component A), and keep from light.
   Note: The HRP reaction mixture is stable at room temperature for at least 8 hours without loose activity if kept from light.

2. Prepare serially diluted HRP standards (0 to 10 mU/mL):

2.1 20 U/mL HRP stock solution: Add 1 mL of PBS with 0.1% BSA into the vial of Horseradish Peroxidase (Component C).
Note: The unused HRP stock solution should be divided into single use aliquots and stored at -20 °C.

2.2 Add 1 µL of 20 U/mL HRP stock solution (from Step 2.1) in 1999 µL of PBS with 0.1% BSA to get 10 mU/mL HRP standard stock solution.

2.3 Take 200 µL of 10 mU/mL HRP standard stock solution to perform 1:2 serial dilutions to get 5, 2.5, 1.25, 0.625, 0.3, 0.15, 0.075 and 0 mU/mL serially diluted HRP standards.

2.4 Add serially diluted HRP standards and/or HRP-containing test samples into a solid black 96-well microplate as described in Tables 1 and 2.

Table 1 Layout of HRP standards and test samples in a solid black 96-well microplate

BL	BL	TS	TS	….	….						
PS1	PS1	….	….	….	….						
PS2	PS2										
PS3	PS3										
PS4	PS4										
PS5	PS5										
PS6	PS6										
PS7	PS7										

Note: PS= Peroxidase Standards; BL=Blank Control; TS=Test Samples.

Table 2 Reagent composition for each well

HRP Standards	**Blank Control**	**Test Sample**
Serial Dilutions*: 50 µL	PBS with 0.1% BSA: 50 µL	50 µL

Note: Add the serially diluted HRP standards from 0.075 mU/mL to10 mU/mL into wells from PS1 to PS7 in duplicate.

3. Run HRP assay in supernatants:

3.1 Add 50 µL of HRP reaction mixture (from Step 1) into each well of HRP standard, blank control, and test samples (see Step 2.4) to make the total HRP assay volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL of sample and 25 µL of HRP reaction mixture into each well.

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

3.3 Monitor the luminescence intensity by using a standard luminometer.

Data Analysis

The luminescence in blank wells with PBS and 0.1% BSA is used as a control, and subtracted from the values for those wells with HRP reactions. A HRP standard curve is shown in Figure 1.

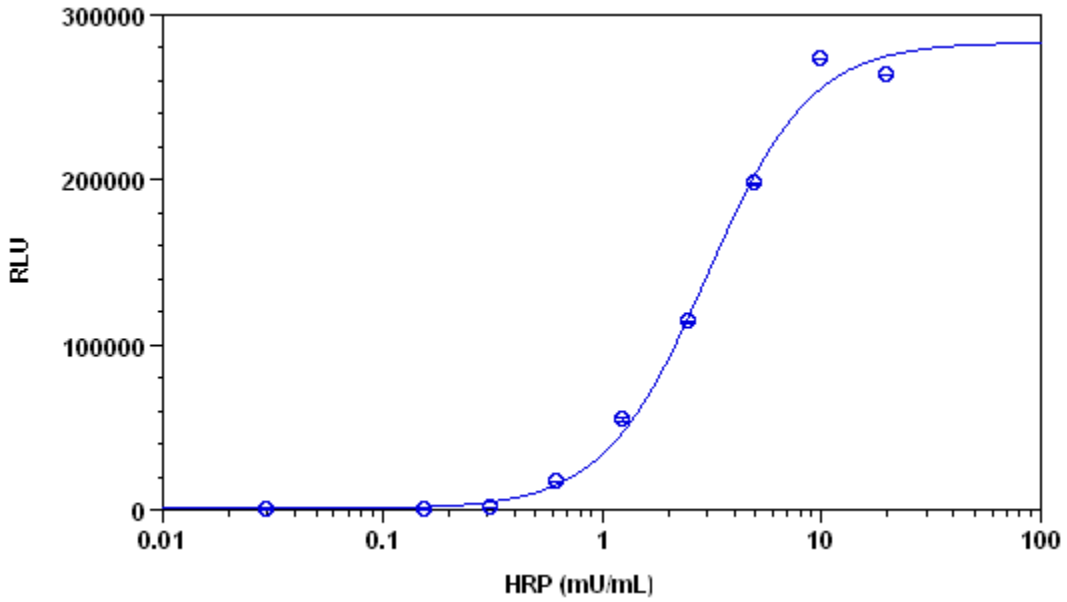

Figure 1. HRP dose response was measured with the Amplite™ Luminometric Peroxidase Assay Kit in a black 384-well plate using a NOVOstar plate reader (BMG Labtech). As low as 150 μU/mL peroxidase can be detected with 30 minutes incubation (n=3).

References

1. Didenko VV, Baskin DS. (2006) Horseradish peroxidase-driven fluorescent labeling ofnanotubes with quantum dots. Biotechniques, 40, 295.
2. Almeida LE, Imasato H, Tabak M. (2006) Enzymatic oxidation of dipyridamole in homogeneous and micellar solutions in the horseradish peroxidase-hydrogen peroxide system. Biochim Biophys Acta, 1760, 216.
3. Krieg R, Halbhuber KJ. (2003) Recent advances in catalytic peroxidase histochemistry. Cell Mol Biol (Noisy-le-grand), 49, 547.
4. Matsui T, Nakayama H, Yoshida K, Shinmyo A. (2003) Vesicular transport route of horseradish C1a peroxidase is regulated by N- and C-terminal propeptides in tobacco cells. Appl Microbiol Biotechnol, 62, 517.
5. Wu TP, Zheng L, Ruan KC. (1998) Effect of Calcium Ion on Conformation of Horseradish Peroxidase Isoenzyme C. Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai), 30, 510.
6. Greco O, Folkes LK, Wardman P, Tozer GM, Dachs GU. (2000) Development of a novel enzyme/prodrug combination for gene therapy of cancer: horseradish peroxidase/indole-3-acetic acid. Cancer Gene Ther, 7, 1414.

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