Amplite™ Luminometric Peroxidase Assay Kit

Luminescence

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

Introduction

Horseradish Peroxidase (HRP) is a small molecule (MW ~40 KD) that is widely used in a variety of biological detections. HRP conjugates are extensively used as secondary detection reagents in ELISAs, immuno-histochemical techniques, Northern, Southern and Western blot analyses. Due to its small size, it rarely causes steric hindrance problem with the antibody/antigen complex formation. It is usually conjugated to an antibody in a 4:1 ratio. Additionally, HRP 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.

We offer this luminometric HRP assay in a one-step, homogeneous, no wash assay system. This kit uses our Amplite™ luminometric HRP substrate to quantify peroxidase in solutions. The kit can be used for ELISAs, characterizing kinetics of enzyme reaction and high throughput screenings, etc. It provides an optimized "mix and read" assay protocol that is compatible with HTS liquid handling instruments. The Kit can detect as low as 100 μU/mL of HRP (Figure1). The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. Its signal can be easily read by a luminescence microplate reader.

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 peroxidase reaction mixture (50 μL) → Add peroxidase standards or test samples (50 μL) → Incubate at room temperature for 30 min to 2 hr → Read luminescent intensity

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

1. Prepare peroxidase reaction mixture: Add 30 μL of 3% stabilized H_2O_2 solution (Component B) to 5 mL of Assay Buffer (Component A), kept from light.

Note: The peroxidase reaction mixture is stable at room temperature for at least 8 hours without loose activity if kept from light.

2. Prepare serial peroxidase (0 to10 mU/mL) standard solutions:

2.1 20 U/mL HRP stock solution: Add 1 mL of PBS with 0.1% BSA into the vial of HRP (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 peroxidase solution.

2.3 Take 200 μL of 10 mU/mL HRP stock solution to perform 1:2 serial dilutions to get 5, 2.5, 1.25, 0.6125, 0.3, 0.15, 0.075 and 0 standard peroxidase solutions.

2.4 Add peroxidase standards and peroxidase-containing test samples into a 96-well solid black microplate as described in Tables 1 and 2.

Table 1. Layout of peroxidase 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

Peroxidase Standard	**Blank Control**	**Test Sample**
Serial dilutions* (50 µL)	PBS with 0.1% BSA: 50 µL	50 µL

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

3. Run HRP assay in supernatants reaction:

3.1 Add 50 µL of peroxidase reaction mixture (from Step 1) to each well of the peroxidase standard, blank control, and test samples (see Step 2.4) 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 peroxidase 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 with a standard luminometer.

Data Analysis

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

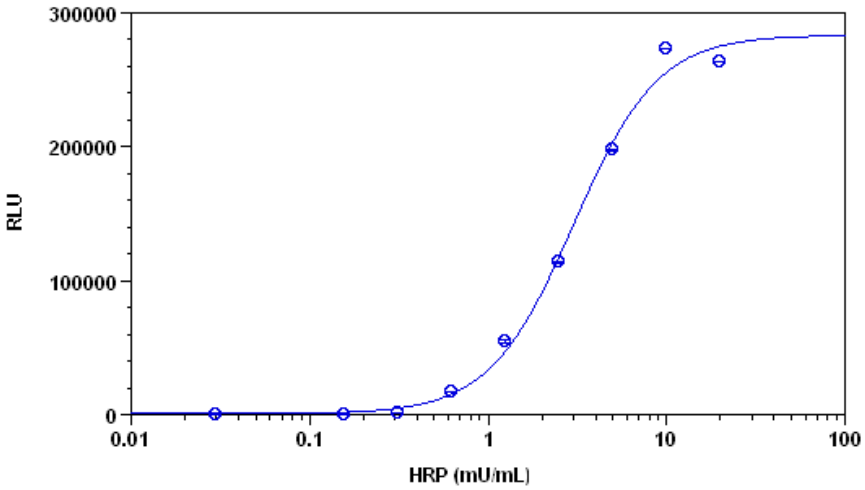

Figure 1. HRP dose response was measured with the Amplite™ Luminometric Peroxidase Assay Kit in a 384-well black plate using a NOVOstar plate reader (BMG Labtech). As low as 150 µU/mL of peroxidase can be detected with 30 minutes incubation time (n=3).