Amplite™ Luminometric Peroxidase (HRP) Assay Kit

Catalog number: 11559
Unit size: 500 Tests

Component	Storage	Amount
Component A: Assay Buffer	Freeze (<-15 °C)	1 bottle (25 mL)
Component B: H2O2	Refrigerate (2-8 °C), Minimize light exposure	1 vial (3% stabilized solution, 200 µL)
Component C: Horseradish Peroxidase	Freeze (<-15 °C), Minimize light exposure	1 vial (20 units)

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. 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 working solution (50 µL)
2. Add HRP standards and/or test samples (50 µL)
3. Incubate at room temperature for 30 minutes to 2 hours
4. Monitor luminescent intensity

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

KEY PARAMETERS

Instrument: Luminescence microplate reader
Recommended plate: Solid white

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. HRP stock solution (20 U/mL):
Add 1 mL of PBS with 0.1% BSA into the vial of Horseradish Peroxidase (Component C).

PREPARATION OF STANDARD SOLUTION

HRP standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11559

Add 1 µL of 20 U/mL HRP stock solution in 1999 µL of PBS with 0.1% BSA to get 10 mU/mL HRP standard solution (PS7). Then use 10 mU/mL standard solution and perform 1:2 serial dilutions to obtain remaining serially diluted standards (PS6-PS1).

PREPARATION OF WORKING SOLUTION

Add 30 µL of 3% stabilized H_2O_2 solution (Component B) into 5 mL of Assay Buffer (Component A) to make HRP working solution and keep from light.

Note The HRP working solution is stable at room temperature for at least 8 hours without activity loss if kept from light.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of HRP standards and test samples in a solid black 96-well microplate. PS=Peroxidase Standards (PS1-PS7, 0.156 to 10 mU/mL), BL=Blank Control, TS=Test Samples.

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

Table 2. Reagent composition for each well.

Well	Volume	Reagent
PS1 - PS7	50 µL	Serial Dilution (0.156 to 10 mU/mL)
BL	50 µL	PBS with 0.1% BSA
TS	50 µL	test sample

1. Prepare HRP standards (PS), 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 minutes to 2 hours, protected from light.

4. Monitor the luminescence intensity by using a standard luminometer.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RLU) 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 Four Parameter Logistics Calculator which can be found at:

https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator

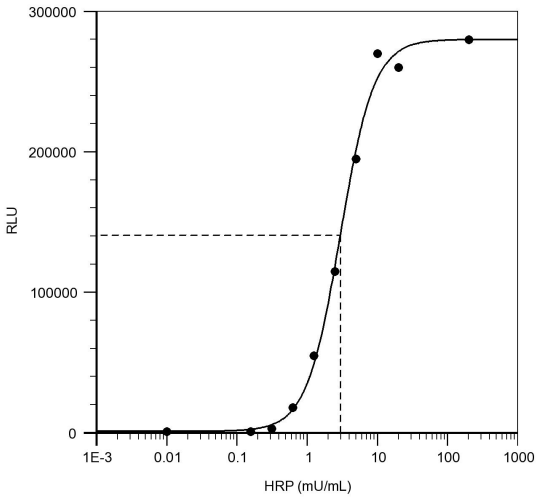

Figure 1. HRP dose response was measured with Amplite™ Luminometric Peroxidase Assay Kit in a solid black 384-well plate using a NOVOstar plate reader (BMG Labtech).

DISCLAIMER