

Amplite™ Fluorimetric Peroxidase Assay Kit

Near Infrared Fluorescence

Ordering Information:	Storage Conditions:	Instrument Platform:
Product Number: 11553 (500 assays)	Keep in freezer Avoid exposure to light	Fluorescence 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, immunohistochemical 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 quick HRP assay in a one-step, homogeneous, no wash assay system. Our Amplite™ Peroxidase Assay Kit uses Amplite™ IR, our near infrared fluorescent HRP substrate to quantify peroxidase in solution. Amplite™ IR generates a substance that has maximum absorption of 647 nm with maximum emission at 670 nm. This near infrared absorption and fluorescence minimize the assay background that is often caused by the autoabsorption and/or autofluorescence 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 screenings, etc. The kit provides an optimized “mix and read” assay protocol that is compatible with HTS liquid handling instruments. It can detect as low as 1 mU/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 either a fluorescence microplate reader at Ex/Em = 600 to 650/650 to 690 nm (maximum Ex/Em = 640 /680 nm) or an absorbance microplate reader at 647±5 nm.

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 1 mU/mL of 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: Amplite™ IR Peroxidase Substrate	1 vial
Component B: H ₂ O ₂	1 vial (3% stabilized solution, 200 µL)
Component C: Assay Buffer	1 bottle (100 mL)
Component D: Horseradish Peroxidase	1 vial (20 units)
Component E: DMSO	1 vial (1 mL)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare peroxidase reaction mixture (50 μ L) \rightarrow Add peroxidase standards or test samples (50 μ L) \rightarrow Incubate at room temperature for 30-60 minutes \rightarrow Read fluorescence intensity at Ex/Em = 640/ 680 nm

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

1. Prepare stock solutions:

- 1.1 Amplite™ IR Peroxidase Substrate stock solution (100X): Add 250 μ L of DMSO (Component E) into the vial of Amplite™ IR Peroxidase Substrate (Component A). The stock solution should be used promptly, and any remaining solution should be aliquoted and refrozen at -20 °C.
Note: Avoid repeated freeze-thaw cycles.

- 1.2 20 U/mL HRP stock solution: Add 1 mL of Assay Buffer (Component C) into the vial of Horseradish Peroxidase (Component D).
Note: The unused HRP solution should be divided into single use aliquots and stored at -20°C.

- 1.3 20 mM H₂O₂ stock solution: Add 22.7 μ L of 3% H₂O₂ (0.88 M, Component B) into 977 μ L of Assay Buffer (Component C).
Note: The diluted H₂O₂ solution is not stable. The unused portion should be discarded.

2. Prepare peroxidase reaction mixture:

Prepare the peroxidase reaction mixture according to the following table and keep from light:

Table 1. Peroxidase Reaction mixture for one 96-well plate (2X)

Components	Volume
Amplite™ IR Peroxidase Substrate stock solution (100X, from Step 1.1)	50 μ L
20 mM H ₂ O ₂ stock solution (from Step 1.3)	50 μ L
Assay Buffer (Component C)	4.9 mL
Total volume	5 mL

3. Prepare serial peroxidase (0 to 300 mU/mL) standard solutions:

Warning 1: The component A is unstable in the presence of thiols such as DTT and β -mercaptoethanol. Thiols higher than 10 μ M (final concentration) would significantly decrease the assay dynamic range.
2: NADH and glutathione (reduced form: GSH) may interfere with the assay.

- 3.1 Add 15 μ L of 20 U/mL HRP stock solution (from Step 1.2) into 985 μ L of Assay Buffer (Component C) to get 300 mU/mL peroxidase solution.
- 3.2 Take 200 μ L of 300 mU/mL HRP stock solution to perform 1:3 serial dilutions to get 100, 30, 10, 3, 1, 0.3 and 0 mU/mL standard peroxidase solutions.
- 3.3 Add peroxidase standards and peroxidase-containing test samples into a 96-well solid black microplate as described in Tables 2 and 3.

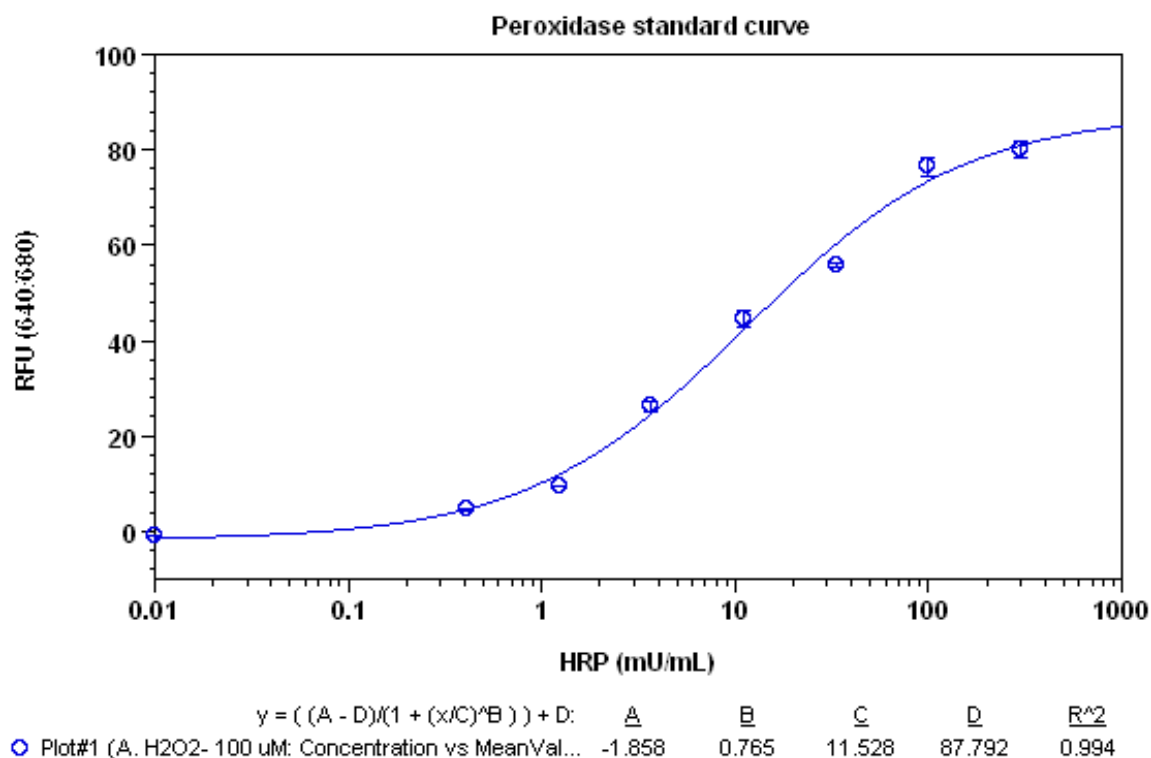


Figure 1. HRP dose response was measured with the Amplite™ Fluorimetric Peroxidase Assay Kit in a 384-well black plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 1 mU/mL of peroxidase can be detected with 30 minutes incubation time (n=3).

References

1. Porstmann, B., Porstmann, T., Nugel, E. and Evers, U. (1985). Which of the commonly used marker enzymes gives the best results in colorimetric and fluorimetric enzyme immunoassays: horseradish peroxidase, alkaline phosphatase, β-galactosidase? *J. Immunol. Meth.* 79, 27-37.
2. Wordinger, R.J., Miller, G.W. and Nicodemus, D.S. (1987). *Manual of Immunoperoxidase Techniques, 2nd Edition*. Chicago: American Society of Clinical Pathologists Press, pp. 23-24.
3. Yolken, R.H. (1982). Enzyme immunoassays for the detection of infectious antigens in body fluids: current limitations and future prospects. *Rev. Infect. Dis.* 4(1), 35-68.
4. Cordell, J.L., et al. (1984). Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). *J. Histochem. Cytochem.* 32, 219-229.
5. Passey, R.B., et al. (1977). Evaluation and comparison of 10 glucose methods and the reference method recommended in the proposed product class standard. *Clin. Chem.* 23(1), 131.
6. Hosoda, H., Takasaki, W., Tsukamoto, R. and Nambara, T. (1987). Sensitivity of steroid immunoassays. Comparison of alkaline phosphatase, β-galactosidase and horseradish peroxidase as labels in a colorimetric assay system. *Chem. Pharm. Bull.* 35, 3336-3342.
7. Samoszuk, M.K., et al. (1989). *Antibody, Immunoconjugates and Radiopharmaceuticals* 2, 37-46.

Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.