Amplite™ Fluorimetric Goat Anti-Rabbit IgG-HRP ELISA Assay Kit

Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 11541 (10 plates)	Different storage conditions are required.	Fluorescence microplate readers Absorbance 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 as well as Northern, Southern and Western blot analyses. Due to its small size, HRP rarely causes any steric hindrance problem with the antibody/antigen complex formation. In addition, HRP conjugates are much more stable than other enzyme conjugates, making the HRP-based ELISA assays much more robust.

This kit uses our outstanding Amplite™ Red HRP substrate. Colorless and non-fluorescent Amplite™ Red generates an intense red fluorescent product upon reaction with hydrogen peroxide in the presence of HRP. The increase in fluorescence intensity is proportional to HRP activity in the presence of H_2O_2.

Our Amplite™ Fluorimetric ELISA Assay Kit can be used for the assays in which goat anti-rabbit IgG is served as the secondary detection agent. It provides an optimized assay protocol that is compatible with HTS liquid handling instruments. As little as 3 ng/well of a polyclonal antibody can be detected. Its signal can be easily read by either a fluorescence microplate reader at Ex/Em = ~540/590 nm or an absorbance microplate reader at ~576 nm.

Kit Key Features

Sensitive:	Detect as low as 3 ng/well of total rabbit IgG.
Continuous:	Can be 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	Storage
Component A: Amplite™ Red Peroxidase Substrate	2 vials	-20 °C
Component B: H_2O_2	1 vial (3% stabilized solution, 500 µL)	4 °C
Component C: Assay Buffer	1 bottle (100 mL)	4 °C
Component D: DMSO	1 vial (1 mL)	4 °C
Component E: Goat Anti-Rabbit IgG-HRP Conjugate	1 vial (25 µL)	4 °C

Assay Protocol for One Plate

Brief Summary

Prepare ELISA plate → Prepare peroxidase reaction mixture → Add 100 µL/well peroxidase reaction mixture into ELISA plate → Incubate at room temperature for 15-60 minutes → Monitor fluorescence intensity at Ex/Em = 540/590 nm

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

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1. Prepare ELISA plate:

1.1 Prepare ELISA microplate (including appropriate controls): Perform all necessary ELISA preparation steps.

1.2 Make goat anti-rabbit IgG-HRP conjugate working solution: Add 2 µL of goat anti-rabbit IgG-HRP conjugate (Component E) into 10 mL of PBS with 1% BSA (PBS-BSA, not included).
Notes 1: 10 mL of goat anti-rabbit IgG-HRP conjugate working solution is enough for 1 plate. The concentration of this goat anti- rabbit IgG-HRP conjugate working solution is recommended as an initial concentration to try.
2: The optimal concentration for each particular application may need to be determined empirically.

1.3 Wash the ELISA wells three times with PBS containing 0.02% to 0.05% Tween® 20 (PBS-Tween) and drain.

1.4 Add 100 µL of the diluted goat anti-rabbit IgG-HRP conjugate working solution (from Step 1.2) into each well (from Step 1.3)

1.5 Incubate at room temperature for 30 minutes. Drain off the HRP conjugate.

1.6 Wash the wells three times with PBS-Tween and drain.

2. Prepare stock solutions:

2.1 200X Amplite™ Red peroxidase substrate stock solution: Add 250 µL of DMSO (Component D) into the vial of Amplite™ Red Peroxidase Substrate (Component A). The 200X Amplite™ Red peroxidase substrate stock solution should be used promptly. Any remaining solution should be aliquoted and refrozen at -20 °C.
Note: 50 µL of the 200X Amplite™ Red peroxidase substrate stock solution is enough for 1 plate. Aliquot and store unused 200X Amplite™ Red peroxidase substrate stock solution at -20 °C. Avoid repeated freeze-thaw cycles.

2.2 20 mM H_2O_2 stock solution: Add 22.7 µL of 3% H_2O_2 (0.88 M, Component B) into 977µL of Assay Buffer (Component C).
Note: The diluted H_2O_2 stock solution is not stable. The unused portion should be discarded.

3. Prepare peroxidase reaction mixture:

Prepare peroxidase reaction mixture according to Table 1 and keep from light.

Table 1. Proxidase reaction mixture for one 96-well plate (1X)

Components	**Volume**
Amplite™ Red peroxidase substrate stock solution (200X, from Step 2.1)	50 µL
20 mM H_2O_2 stock solution (from Step 2.2)	100 µL
Assay buffer (Component C)	9.85 mL
Total volume	10 mL

4. Run peroxidase assay in ELISA plate:

4.1 Add 100 µL of peroxidase reaction mixture (from Step 3) into each drained microplate well containing the samples and controls (from Step 1.6).

4.2 Incubate the reaction at room temperature for 30 minutes or longer, protected from light.

4.3 Monitor the fluorescence increase with a fluorescence plate reader at excitation 530-570 nm (optimal at 540 nm) and emission 590-600 nm.
Note: The plate can also be read by an absorbance microplate reader at the wavelength of 576±5 nm. The absorption detection has lower sensitivity compared to fluorescence reading.

Data Analysis

For each sample, correct for background fluorescence or absorbance by subtracting the values derived from the negative.

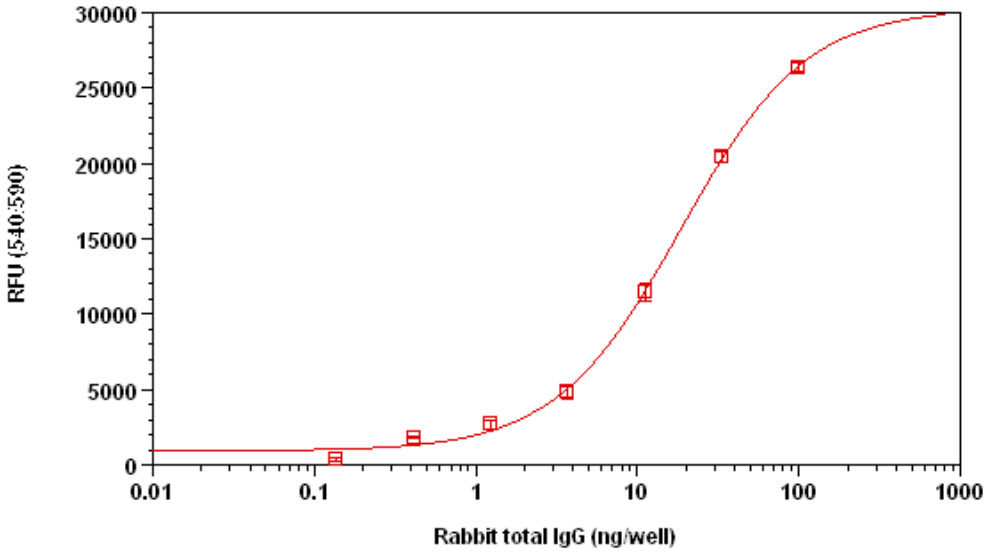

Figure 1. Detection of total rabbit IgG using the Amplite™ Fluorimetric ELISA Kit. Rabbit IgG was diluted into 1 µg/mL and made 1 to 3 serial dilutions in 0.2 M sodium bicarbonate buffer at pH 9.4. 100 µL/well serial dilutions were coated into a black 96-well plate at 4 °C overnight, and blocked with 3% milk in PBS and 0.02% Tween at 4 °C overnight. The wells were washed, and assayed using the reagents. 1 to 6000 dilutions of goat anti-rabbit IgG-HRP conjugate were used. The reactions were incubated for 15 to 60 minutes and then measured for fluorescence at Ex/Em = 540/590 nm with Gemini fluorescence microplate reader (Molecular Devices). As low as 3 ng/well of total rabbit IgG can be detected with 30 minutes incubation (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, ß-galactosidase? *J. Immunol. Meth.* 79**,** 27-37.
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3. 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.
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5. 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.