Amplite™ Fluorimetric Goat Anti-Mouse IgG-HRP Conjugate ELISA Assay Kit *Red Fluorescence*

Catalog number: 11540
Unit size: 1000 Tests

Component	Storage	Amount
Component A: Amplite™ Red Peroxidase Substrate	Freeze (<-15 °C), Minimize light exposure	2 vials
Component B: H2O2	Refrigerate (2-8 °C), Minimize light exposure	1 vial (3% stabilized solution, 500 µL)
Component C: Assay Buffer	Freeze (<-15 °C)	1 bottle (100 mL)
Component D: DMSO	Freeze (<-15 °C)	1 vial (1 mL)
Component E: Goat Anti-Mouse IgG-HRP Conjugate	Freeze (<-15 °C)	1 vial (25 µL)

OVERVIEW

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 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. Our Amplite™ Fluorimetric ELISA Assay Kit contains all the essential components including our fluorogenic Amplite™ Red HRP substrate for ELISA detection. The kit provides an optimized assay protocol that is compatible with HTS liquid handling, as little as 10 pg of a monoclonal antibody/well of a microplate can be detected. Its signal can be easily read by either fluorescence microplate reader or absorbance microplate reader. It can be used for assays that detect goat anti-mouse IgG as the secondary detection agent.

AT A GLANCE

Protocol summary

1. Prepare ELISA plate
2. Prepare peroxidase working solution
3. Add 100 µL/well of peroxidase working solution into the ELISA plate
4. Incubate at room temperature for 15 - 60 minutes
5. Monitor fluorescence intensity at Ex/Em = 540/590 nm

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Solid black

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. Amplite™ Red Peroxidase Substrate stock solution (200X):
Add 250 µL of DMSO (Component D) into the vial of Amplite™ Red Peroxidase Substrate (Component A).

Note 50 µL of the Amplite™ Red peroxidase substrate stock solution is enough for 1 plate.

2. H_2O_2 stock solution (20 mM):
Add 22.7 µL of 3% H_2O_2 (0.88 M, Component B) into 977 µL of Assay Buffer (Component C).

Note The diluted H_2O_2 stock solution is not stable. The unused portion should be discarded.

PREPARATION OF STANDARD SOLUTION

Mouse IgG standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11540

Mouse IgG (not included) can be used to generate a standard curve. Dilute total mouse IgG with 0.2 M sodium bicarbonate buffer (pH 9.4) into microplate, using an initial concentration of 3 ug/mL and 1:3 dilution factor.

PREPARATION OF WORKING SOLUTION

1. Goat anti-mouse IgG-HRP conjugate working solution:
Add 2 µL of goat anti-mouse IgG-HRP conjugate (Component E) to 10 mL of PBS with 1% BSA (PBS-BSA, not included).

Note 10 mL of goat anti-mouse IgG-HRP conjugate working solution is enough for 1 plate. The concentration of this goat anti-mouse IgG-HRP conjugate working solution is recommended as an initial concentration to try. The optimal concentration for each particular application should be determined empirically.

2. Peroxidase working solution:
Add 50 µL of Amplite™ Red Peroxidase Substrate stock solution (200X) and 100 µL of H_2O_2 stock solution (20 mM) into 9.85 mL of Assay Buffer (Component C) to make a total volume of 10 mL Peroxidase working solution. Keep from light.

SAMPLE EXPERIMENTAL PROTOCOL

Prepare ELISA plate:

1. Perform all necessary ELISA preparation steps.

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

3. Add 100 µL of prepared goat anti-mouse IgG-HRP conjugate working solution into each well.

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

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

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Run peroxidase assay in ELISA plate:

1. Add 100 µL of peroxidase working solution into each drained microplate well containing the samples and controls.

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

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. However, the absorption detection has lower sensitivity compared to fluorescence reading.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) 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 Mouse Total IgG 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

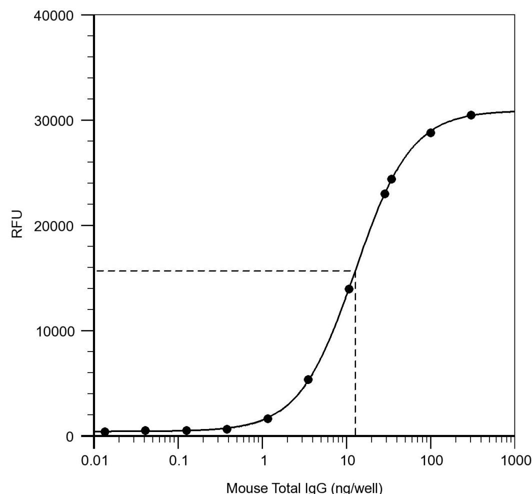

Figure 1. Detection of mouse total IgG using the Amplite™ Fluorimetric Goat Anti-Mouse IgG-HRP ELISA Kit. Mouse IgG was diluted into 3 µg/mL and made 1 to 3 serial dilutions in 0.2 M sodium bicarbonate buffer, pH 9.4. 100 µL/well serial dilutions were coated into a solid black 96-well plate at 4 °C overnight, and blocked with 3% milk in PBS and 0.02% Tween-20 at 4 °C overnight. The wells were washed and assayed by using the reagents. 1 to 5000 dilutions of goat anti-mouse IgG-HRP conjugate were used. The reactions were incubated for 10 to 60 minutes and then measured for fluorescence at Ex/Em = 540/590 nm using Gemini fluorescence microplate reader (Molecular Devices).

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