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

Catalog number: 11541
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-Rabbit 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 Polyclonal antibody in the 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 the assays that detect goat anti-rabbit IgG as the secondary detection agent.

AT A GLANCE

Protocol summary

1. Prepare ELISA plate
2. Add Goat Anti-Rabbit IgG-HRP Conjugate working solution (100 µL/well)
3. Incubate at room temperature for 30 minutes
4. Wash the wells (3X with PBS-Tween)
5. Add Peroxidase working solution (100 µL/well)
6. Incubate at room temperature for 30 - 60 minutes
7. Monitor fluorescence intensity at Ex/Em = 540/490 nm (Cutoff = 570 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
Instrument:	Absorbance microplate reader
Absorbance:	576 ± 5 nm
Recommended plate:	Clear bottom

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) and mix well to make 200X Amplite™ Red Peroxidase Substrate stock solution.

Note 50 µL of 200X Amplite™ Red Peroxidase Substrate stock solution is enough for 1 plate. The stock solution should be used promptly. Protect from light.

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

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

PREPARATION OF WORKING SOLUTION

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

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

Prepare ELLISA plate:

1. Prepare ELISA microplate (including appropriate controls) by performing all necessary ELISA preparation steps.

2. Add 2 µL of Goat Anti-Rabbit IgG-HRP Conjugate (Component E) into 10 mL of PBS with 1% BSA (PBS-BSA, not included) to make Goat Anti-Rabbit IgG-HRP Conjugate working solution.

   Note 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. The optimal concentration for each particular application may need to be determined empirically.

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

4. Add 100 µL of Goat Anti-Rabbit IgG-HRP Conjugate working solution into each well.

5. Incubate at room temperature for 30 minutes. Drain off the Goat Anti-Rabbit IgG-HRP Conjugate.

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

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, Emission = 590-600 nm (optimal Ex/Em = 540/590 nm, Cutoff = 570 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.

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 Rabbit 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

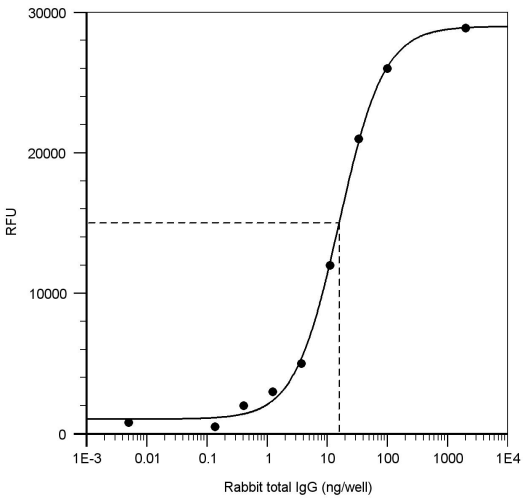

Figure 1. Detection of rabbit total IgG using the Amplite™ Fluorimetric ELISA Kit. Rabbit IgG was diluted into 1 µg/mL and made 1 to 3 serial dilutions in 0.2 M sodium bicarbonate buffer at pH 9.4. 100 µL/well serial dilutions were coated into a black 96-well plate at 4°C overnight, and blocked with 3% milk in PBS and 0.02% Tween 20 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).

DISCLAIMER