SensoLyte® ADHP Horseradish Peroxidase ELISA Assay Kit *Fluorimetric*

Catalog #	71110-M
Kit Size	500 Assays (96-well plate)

- ***Convenient Format:*** Complete kit including all the assay components.
- ***Optimized Performance:*** Optimal conditions for the detection of generic protease activity.
- ***Enhanced Value:*** Less expensive than the sum of individual components.
- ***High Speed:*** Minimal hands-on time.
- ***Assured Reliability:*** Detailed protocol and references are provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	ADHP, fluorogenic peroxidase substrate	250 µL
Component B	H_2O_2	1 vial
Component C	Assay buffer	60 mL
Component D	Horseradish peroxidase-conjugated goat anti-mouse IgG	30 µL

Other Materials Required (but not provided)

- Required ELISA microplate: Black, flat-bottom, 96-well plate with high-binding surface.
- Fluorescence microplate reader: Capable of detecting emission at 590 nm with excitation at 530-560 nm.

Storage and Handling

- Store all components at -20°C.
- For convenience, the Component C can be stored at room temperature

Introduction

Horseradish peroxidase (HRP) conjugated secondary antibody and streptavidin are widely used as the secondary detection systems in ELISA. The SensoLyte® ADHP Peroxidase Assay Kit uses ADHP (10-Acetyl-3, 7-dihydroxyphenoxazine) as a fluorogenic substrate for HRP. ADHP (also called Amplex Red Reagent) is considered to be the most stable and sensitive fluorogenic substrate for HRP. It has been used to detect as low as 10^{-15} M concentration of target protein in an ELISA format. Its signal can be easily read by a fluorescence microplate reader at Ex/Em=530-560 nm/590 nm.

Protocol

Note 1: Prepare the ELISA assay plate according to standard ELISA procedures (refer to **Appendix**). HRP conjugated goat anti-mouse IgG (Component D) is provided in this kit.
Note 2: Warm up all kit components to room temperature when the ELISA plate is ready for detection.

1. Prepare stock solutions.

1.1 H_2O_2 stock solution: Add 100 µL of deionized water into the H_2O_2 vial (Component B). Store this stock solution tightly capped at 4°C.

2. Prepare ADHP reaction mixture.

2.1 Prepare fresh H_2O_2 solution (100X) by adding 4 µL of H_2O_2 stock solution into 196µL of reaction buffer.
Note: Diluted H_2O_2 solution (100X) is not stable. The unused portion should be discarded.

2.2 Prepare the ADHP reaction mixture according to the following Table. Protect prepared reaction mixture from light.

Table 1. ADHP Reaction mixture for one 96-well plate (100 assays).

Components	Volume
ADHP (Component A)	50 µL
H_2O_2 solution (100X)	100 µL
Assay buffer (Component C)	9.85 mL
Total volume	10 mL

3. Start the HRP reaction.

3.1 Add 100 µL of ADHP reaction mixture prepared in Step 2.2 to each ELISA microplate well.
3.2 Incubate the reaction for 15 to 30 minutes. Protect the reaction from light.
3.3 Read the plate using a fluorescence microplate reader with a filter set of Ex/Em=530-560 nm/590 nm.

Appendix: General ELISA protocol

1. Required buffers:

1. Coating buffer: 1.59 g of Na_2CO_3 and 2.93 g of $NaHCO_3$ in 1L of deionized H_2O. pH is 9.6 without adjustment.
2. Phosphate-buffered saline (PBS): 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4, and 0.24 g of KH_2PO_4 in 800 ml of deionized H_2O. Adjust pH to 7.2-7.4 with HCl or NaOH. Add H_2O to 1L.
3. Blocking buffer: add 10 g of bovine serum albumin (BSA) and 0.2 mL of Tween®-20 into 1 L of PBS.
4. EIA buffer: add 1 g of bovine serum albumin (BSA) and 0.2 mL of Tween®-20 into 1 L of PBS.
5. Wash buffer: add 0.2 mL of Tween®-20 into 1 L of PBS.

2. ELISA:

1. Coating: Add 100 µL of capture antibody to each well of the 96-well plate at a concentration of 2-10 µg/mL in coating buffer. Seal the plate with plate sealer and incubate at 4°C overnight.
2. Washing: Discard the solution and wash the plate with 200 µL of wash buffer per well three to five times. Soak the plate during the last wash step for 5 min. Pad dry on paper towel.
3. Blocking: Add 200 µL of blocking buffer and incubate 1h at room temperature.
4. Washing: Repeat Step 2.
5. Add sample: Dilute sample to be tested in EIA buffer to an appropriate concentration. Add 100 µL of the diluted sample to each well and incubate at room temperature for 1h on a plate shaker.
6. Washing: Repeat Step 2.
7. Add detection antibody: Dilute mouse detection antibody in EIA buffer to the appropriate concentration. Add 100 µL of diluted antibody to each well and incubate at room temperature for 1h on a plate shaker.
8. Washing: Repeat Step 2.
9. Add secondary antibody: Dilute HRP-conjugated goat anti-mouse antibody (Component D) in EIA buffer to the appropriate concentration (1:5,000 to 1:100,000 dilution). Add 100 µL of diluted antibody to each well and incubate at room temperature for 1h on a plate shaker.
10. Washing: Repeat Step 2.
11. Detection by substrate: The plate is now ready for the ADHP detection (refer to Protocol)