



SensoLyte[®] ADHP Peroxidase Assay Kit **Fluorimetric**

Revision# 1.2	Last Updated: July 2021
Catalog #	AS-71111
Kit Size	500 Assays (96-well plate)

- **Convenient Format:** Complete kit includes all the assay components.
- **Optimized Performance:** Optimal conditions for detecting peroxidase.
- **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	10 mM, 250 μ L
Component B	H ₂ O ₂	1 vial
Component C	Assay buffer	60 mL

Other Materials Required (but not provided)

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

Storage and Handling

- Store Component A at -20°C and keep from direct light.
- Store the rest of the components at 4°C

Introduction

Cellular peroxidases play an important role in protecting cell from oxidative injury. Horseradish peroxidase (HRP) conjugates are extensively used as secondary detection reagents in ELISA.

The SensoLyte® ADHP Peroxidase Assay Kit provides a convenient, highly sensitive fluorescent assay for detecting peroxidase activity in solution, cell extract, and ELISA. Non-fluorescent ADHP (10-Acetyl-3, 7-dihydroxyphenoxazine) can be oxidized to the strongly fluorescent resorufin in presence of H₂O₂ by peroxidases, such as horseradish peroxidase (HRP), eosinophil peroxidase¹ and myeloperoxidase.¹ Resorufin signal (Ex/Em=530-560nm/590nm) can easily be read with a fluorescence microplate reader.

Protocol

Note 1: Warm all kit components to room temperature before starting the experiment.

Note 2: Choose Protocol A or B based on your needs.

Protocol A. Detecting peroxidase activity in biological samples

1. Prepare stock solution (for first time use only).

- 1.1 H₂O₂ stock solution: Add 100 µL of deionized water into one vial of H₂O₂ (Component B). Store this stock solution tightly capped at 4°C.

2. Prepare peroxidase-containing samples.

- 2.1 Add 50 µL/well of samples to 96-well plate.

Note: Exceeding large amount of peroxidase may further convert fluorescent resorufin to non-fluorescent resazurin and lead to reduce fluorescent signal. It is necessary to test first with different sample dilutions.

3. Prepare ADHP reaction mixture.

- 3.1 Prepare ADHP reaction mixture fresh according to the following Table and keep from light.

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

Components	Volume
ADHP (Component A)	50 µL
H ₂ O ₂ stock solution (Component B)	10 µL
Assay buffer (Component C)	4.94 mL
Total volume	5 mL

4. Initiate the enzymatic reaction:

- 4.1 Add 50 µL/well of ADHP reaction mixture. Mix the reagents by gently shaking plate for 30 sec.

- 4.2 Measure signals:

- For kinetic reading: Immediately start measuring fluorescence, Ex/Em=530-560 nm/590 nm. Record data every 5 min. for 15 to 30 min.
- For end-point reading: Incubate reaction at the desired temperature for 15-30 min, then measure fluorescence, Ex/Em=530-560 nm/590 nm.

Protocol B. Detecting horseradish peroxidase (HRP) activity in ELISA

Note: For the preparation of ELISA plate, please refer to Appendix I.

1. Prepare stock solution (for first time use only).

- 1.1 H₂O₂ stock solution: Add 100 μ L of deionized water into one vial of H₂O₂ (Component B). Store this stock solution tightly capped at 4°C.

2. Prepare ADHP reaction mixture.

- 2.1 Prepare ADHP reaction mixture fresh according to the following Table and keep from light.

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

Components	Volume
ADHP (Component A)	50 μ L
H ₂ O ₂ stock solution (Component B)	2 μ L
Assay buffer (Component C)	10 mL
Total volume	10 mL

3. Detect HRP activity.

- 3.1 Add 100 μ L/well of ADHP reaction mixture. Mix the reagents by gently shaking the plate for 30 sec.
- 3.2 Measure signal: Incubate reaction at the desired temperature for 15-30 min, then measure fluorescence, Ex/Em=530-560 nm/590 nm.

Appendix: General ELISA protocol

1. Required buffers:

1. Coating buffer: 1.59 g of Na₂CO₃ and 2.93 g of NaHCO₃ in 1L of deionized H₂O. pH is 9.6 without adjustment.
2. Phosphate-buffered saline (PBS): 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na₂HPO₄, and 0.24 g of KH₂PO₄ in 800 ml of deionized H₂O. Adjust pH to 7.2-7.4 with HCl or NaOH. Add H₂O 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 Tween[®]-20 into 1 L of PBS.
5. Wash buffer: Add 0.2 mL of Tween[®]-20 into 1 L of PBS.

2. Required ELISA microplate:

Use black high-binding ELISA plates for better signal to noise ratio.

3. ELISA procedures.

1. Coating: Add 100 µL of peptide-conjugate (PP-BSA) to each well of the 96-well plate at a concentration of 10 µg/mL in coating buffer. Seal plate with Para film[®] and incubate at 4°C overnight.
2. Washing: Discard solution and wash plate with 300 µL of wash buffer per well three to five times. Soak plate during the last wash step for 5 min. Pat dry on paper towel.
3. Blocking: Add 200 µL/well of blocking buffer and incubate for 1h at room temperature.
4. Washing: Repeat Step 2.
5. Add the primary antibody: Dilute anti-peptide antibody in EIA buffer to an appropriate concentration. Add 100µL/well of the diluted antibody and incubate at room temperature for 1h on a plate shaker.
6. Washing: Repeat Step 2.
7. Add the secondary antibody: Dilute HRP conjugated secondary antibody in EIA buffer to an appropriate concentration (1:5000 to 1:100,000 dilution). Add 100 µL/well of diluted secondary antibody and incubate at room temperature for 1h on a plate shaker.
8. Washing: Repeat Step 2.
9. Detection: The plate is now ready for the ADHP detection (refer to Protocol B).

References

1. Mohanty, JG. et al. *J. Immunol. Methods* **202**, 133 (1997).