

# SensoLyte® ADHP Peroxidase Assay Kit \*Fluorimetric\*

Revision Number:1.1	Last Revised: October 2014		
Catalog #	AS-71111		
Kit Size	500 Assays (96-well) or 1250 Assays (384-well)		

- *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 μL
Component B	$H_2O_2$	1 vial
Component C	Assay buffer	60 mL

## Other Materials Required (but not provided)

- Microplate: Black, flat-bottom, 96-well plate.
- <u>Fluorescence microplate reader:</u> 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  $H_2O_2$  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  $\underline{\text{H}_2\text{O}_2}$  stock solution: Add 100 μL of deionized water into one vial of  $\underline{\text{H}_2\text{O}_2}$  (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 or 20 μL/well to 384-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.

<u>3.1</u> Prepare ADHP reaction mixture fresh according to the following Table and keep from light.

Table 1	ADHP 1	reaction	mivture	for one	96-well	nlate	(100 assays	

Components	Volume
ADHP (Component A)	50 μL
H <sub>2</sub> O <sub>2</sub> 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 (96-well plate) or 20 μL/well (384-well plate) of ADHP reaction mixture. Mix the reagents by gently shaking plate for 30 sec.
- 4.2 Measure signals:
  - <u>For kinetic reading:</u> Immediately start measuring fluorescence, Ex/Em=530-560 nm/590 nm. Record data every 5 min. for 15 to 30 min.
  - <u>For end-point reading:</u> 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  $\underline{\text{H}_2\text{O}_2}$  stock solution: Add 100 μL of deionized water into one vial of  $\underline{\text{H}_2\text{O}_2}$  (Component B). Store this stock solution tightly capped at 4°C.

# 2. Prepare ADHP reaction mixture.

<u>2.1</u> 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 <sub>2</sub> O <sub>2</sub> stock solution (Component B)	2 μL
Assay buffer (Component C)	10 mL
Total volume	10 mL

# 3. Detect HRP activity.

- 3.1 Add 100  $\mu$ L/well (96-well plate) or 50  $\mu$ L/well (384-well plate) 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 Na<sub>2</sub>CO<sub>3</sub> and 2.93 g of NaHCO<sub>3</sub> in 1L of deionized H<sub>2</sub>O. 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<sub>2</sub>HPO<sub>4</sub>, and 0.24 g of KH<sub>2</sub>PO<sub>4</sub> in 800 ml of deionized H<sub>2</sub>O. Adjust pH to 7.2-7.4 with HCl or NaOH. Add H<sub>2</sub>O 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<sup>®</sup>-20 into 1 L of
- 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<sup>®</sup> 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).