SensoLyte® Luminescent Peroxidase Assay Kit **Luminometric**

Revision Number: 1.1	*Last updated: October 2014*

Catalog #	**AS-72127**
Kit Size	500 Assays (96-well) or 1250 Assays (384-well)

- ***Optimized Performance:*** This kit is optimized to detect peroxidase activity.
- ***Enhanced Value:*** It provides enough reagents to perform 500 assays in a 96-well format.
- ***High Speed:*** The entire process can be completed in 30 minutes.
- ***Assured Reliability:*** Detailed protocol is provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	Substrate A	15 mL
Component B	Substrate B	15 mL
Component C	Peroxidase (Horseradish Peroxidase) standard	10 µg/mL, 100 µl
Component D	Assay buffer	60 mL

Other Materials Required (But not Provided)

- 96-well or 384-well microplate: White microplates provide better signal to noise ratio for luminescence reading.
- Luminescent microplate reader

Storage and Handling

- Store all kit components at 4°C.
- Protect Component A from light.

Introduction

Peroxidases are a group of heme-containing enzymes that catalyze oxidation-reduction reactions. Peroxidases use hydrogen peroxide (H_2O_2) or lipid peroxides as substrates for a number of oxidative reactions. Peroxidases are found in bacteria, fungi, plants and animals. Cellular peroxidases play an important role in protecting cell from oxidative injury. Horseradish peroxidase (HRP) is extensively used in ELISA and IHC as a reporter molecule when conjugated with secondary detection reagents.

The SensoLyte® Luminescent Peroxidase Assay Kit detects peroxidase activity in biological samples and in ELISA with HRP-conjugates by using chemiluminescent substrate luminol. Oxidation of luminol by peroxide results in creation of an excited state intermediate product that decays to a lower energy state by releasing photons of light. The assay can detect femtogram range of enzyme (Figure 1).

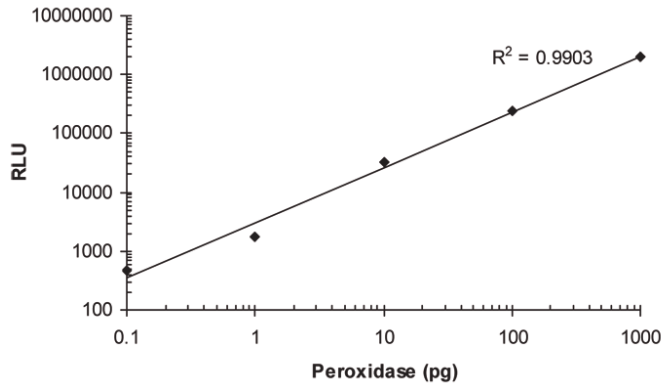

Figure 1. Detection of horseradish peroxidase with the SensoLyte® Luminescent Peroxidase Assay Kit. The detection limit can reach femtogram range (n=3, mean±S.D).

Protocol

Note 1: Warm all kit components to room temperature before starting the experiment.
Note 2: Please use Protocol A or B based on your needs.

Protocol A. Detecting peroxidase activity in biological samples

1. Prepare working solutions.

1.1 Prepare dilutions of peroxidase standard: Dilute peroxidase (10 μg/mL - Component C) to 20 ng/mL (1:500) in assay buffer (Component D). Then make ten-fold serial dilutions to get concentration of 2000, 200, 20, 2, 0.2, 0.02 and 0 pg/mL of enzyme solution.

1.2 Prepare peroxidase substrate mixture: Prepare substrate mixture according to Table 1.

Note: Substrate mixture can be used for 45-60 days if stored properly at 4-8C in a tight capped container.

Table 1. Peroxidase substrate mixture for one 96-well plate (100 assays)

Components	Volume
Substrate A (Component A)	2.5 mL
Substrate B (Component B)	2.5 mL
Total volume	5 mL

2. Set up the enzymatic reaction.

2.1 Add 50 µL (96-well plate) or 20 µL (384-well plate) of biological samples containing peroxidase to the wells.

Note: Large amount of peroxidase may reduce luminescent signal. It is necessary to test a series of sample dilutions to determine the concentrations to use.

2.2 Set up peroxidase standard: Add 50 µL (96-well plate) or 20 µL (384-well plate) serially diluted peroxidase reference solutions to the wells. The final amounts of peroxidase are 1000, 100, 10, 1, 0.1, 0.01, 0.001, and 0 picogram/well (96-well plate).

2.3 Simultaneously establish the following control wells, as deemed necessary:

- Negative control contains 50 µL (96-well plate) or 20 µL (384-well plate) of biological sample without peroxidase.
- Substrate control contains 50 µL (96-well plate) or 20 µL (384-well plate) of assay buffer (Component D).

3. Run the enzymatic reaction.

3.1 Add 50 µL/well (96-well plate) or 20 µL/well (384-well plate) of peroxidase substrate mixture into each well. Mix the reagents completely by shaking the plate gently for 30 sec.

3.2 Measure luminescent signal: The best results can be obtained from 5 to 30 minutes after adding substrate to the sample.

3.3 Data analysis: The luminescence reading from the substrate control well is the background luminescence. This background reading should be subtracted from the readings of the other wells. The luminescence readings are expressed in relative luminescence units (RLU).

Protocol B. Detecting peroxidase activity in ELISA

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

1. Prepare working solutions.

1.1 Prepare peroxidase substrate mixture: Prepare fresh substrate mixture according to Table 1.

Table 1. Peroxidase substrate mixture for one 96-well plate (100 assays)

Components	Volume	
Substrate A (Component A)	2.5 mL	
Substrate B (Component B)	2.5 mL	
Assay Buffer (Component D)	5 mL	5 mL
Total volume	10 mL	

Note: Unused portion of substrate mixture for ELISA should be discarded.

2. Detect peroxidase activity

2.1 Add 100 µl/well (96-well plate) of peroxidase substrate mixture into each well. Shake the plate gently for 30 sec.

2.2 Measure luminescence signal: The best results can be obtained from 5 to 30 minutes after adding substrate to the sample.

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 1 L of deionized H_2O. The pH is 9.6 without adjustment.
2. Phosphate-buffered saline (PBS): 8 g of NaCl, 0.2 g of KCl, 1.44 g Na_2HPO_4, 0.24 g 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 BSA and 0.2 mL of Tween®-20 into 1 L of PBS.
4. EIA buffer: Add 1 g of 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 white or black high-binding ELISA plates for better signal to noise ratio.

3. 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 peroxidase conjugated detection antibody or streptavidin in EIA buffer to the appropriate concentration (1:5000 to 1:100,000 dilution). Add 100 µL of diluted conjugate to each well and incubate at room temperature for 1h on a plate shaker.
8. Washing: Repeat Step 2.
9. Detection by substrate: Plate is now ready for the luminescence detection (refer to the Protocol B).