SensoLyte® Luminescent Peroxidase ELISA Assay Kit **Luminometric**

Revision Number: 1.1	*Last updated: October 2014*

Catalog #	**AS-72128**
Kit Size	500 Assays (96-well)

- ***Optimized Performance:*** This kit is optimized to detect horseradish peroxidase conjugated with antibody or streptavidin in ELISA.
- ***Enhanced Value:*** It provides enough reagents to perform 500 assays in a 96-well format.
- ***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	Assay Buffer	25 mL

Other Materials Required (But not Provided)

- 96-well microplate: White or black ELISA microplates with clear bottom provide better signal to noise ratio for luminescence reading.
- Luminescence 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) as a substrate for a number of oxidative reactions. Horseradish peroxidase (HRP) is extensively used in ELISA and IHC as a reporter molecule when conjugated with secondary detection reagents.

The SensoLyte® Luminescent Peroxidase ELISA Assay Kit is designed to detect HRP conjugated with antibody or streptavidin in ELISA. The chemiluminescent substrate luminol is oxidized by peroxide and creates an excited state intermediate product that decays to a lower energy state by releasing photons of light. The kit provides ample material to perform 500 assays in a 96-well format. The protocol can readily be modified to run assays in a 384-well format.

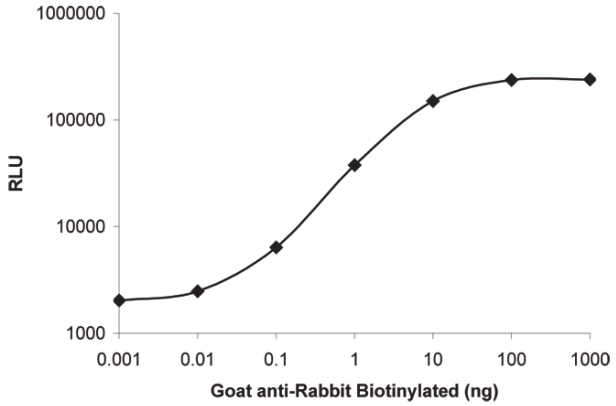

Figure 1. The SensoLyte® Luminescent Peroxidase ELISA Assay Kit was used to detect goat anti-rabbit biotinylated antibody. Wells were coated with total rabbit IgG. After blocking and washing, serially diluted goat anti-rabbit antibody conjugated with biotin was added into the wells, followed by incubation with HRP-labeled streptavidin. The plate was washed again and the peroxidase substrate mixture added to wells. The luminescent signal was measured immediately with microplate reader (Flexstation 384II, Molecular Devices). The assay can detect as low as 1 pg of biotinylated goat-anti-rabbit antibody.

Protocol

Note 1: Prepare an ELISA assay plate according to standard ELISA procedures (refer to the **Appendix**).
Note 2: Warm up all kit components to room temperature when the ELISA plate is ready for detection.

1. Prepare working substrate solution: 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 C)	5 mL	5 mL
Total volume	10 mL	

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

2. Detect HRP activity: Add 100 µl/well of peroxidase substrate mixture into each well. Shake the plate gently for 30 sec, 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 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 dilutions). 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).