Lumi-Phos HRP (PS-atto)

Chemiluminescent Reagent

Product Overview

Catalog Number **PSA- 100**

Contents Solution A - 50 mL
Solution B - 50 mL
For working solution, mix solutions A and B in the ratio of 1:1

Description

Lumi-Phos HRP reagent is recommended primarily for chemiluminescent ELISA detection of proteins bound with antibodies conjugated with horseradish peroxidase (HRP). Reaction of the substrate with an HRP label rapidly generates high-intensity luminescence for sensitive detection in ELISA assays. However, it can also be used for the detection of any HRP-conjugated molecules, proteins or nucleic acids.

Note: Lumi-Phos HRP reagent is invented, developed and manufactured by Lumigen.

Product Characteristics and Applications

- Very rapid onset of chemiluminescence; Peak intensity reached in seconds.
- Excellent sensitivity - less than 10^{-19} moles HRP can be measured.
- Linear calibration curves with slopes of log-log plots equal to 1.0, i.e. one order of magnitude more enzyme yields one order of magnitude more light intensity.

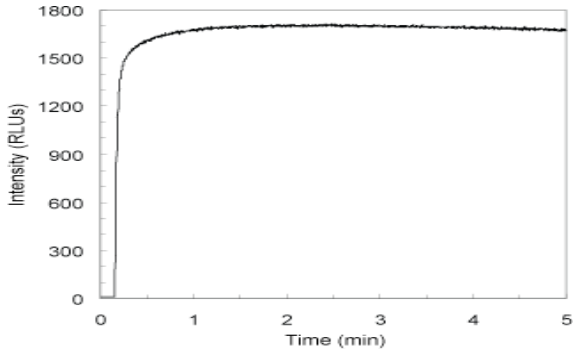

Figure 1: Solution kinetics Lumi Phos-Phos HRP signal intensity over 5 minutes

ELISA Assay – Equipment and Material Required

- White or black high protein binding microtiter plates
- Antigen specific capture antibody
- Antigen and reference set of antigen concentrations
- Antigen specific detection antibody HRP conjugate
- Blocking solution such as 1% BSA
- Washing buffers such as 1X PBS with 0.05% Tween-20
- Lumi-Phos HRP solutions A and B
- Microplate luminometer

Important Notes and Precautions

It is essential that the capture antibody and HRP labeled detection antibody are of high titer and highly specific for the analyte to be detected. The antibodies need to be titrated and tested in ELISA to determine the optimal concentrations for maximum detection sensitivity. The antibody stocks from commercial vendors vary in their binding specificity and protein concentration. As a general rule, antibody stocks may be diluted and used in the range of 10 to 1 µg/mL for capture antibody, and from 0.1 to 0.01 µg/mL for HRP-labeled detection antibody. Other variables that influence the detection sensitivity of the chemiluminescent reagent are the type of microtiter well plate (high or low protein binding), the efficiency of target capture, and the blocking agent used to minimize non-specific background. Maintaining temperature during antibody incubation may help to achieve consistent results.

ELISA Procedure and Chemiluminescent Detection Steps:

1. Coat white or black microtiter wells such as FluoroNunc Maxisorp with 100 µL/well of capture antibody (10 to 1µg/mL in 1X PBS) by incubating for 30 – 60 min. on a shaker platform at room temperature.
2. Wash 3X with 300 µL/well of 1X PBST (with 0.05% Tween-20).
3. Add 300 µL/well of blocking agent (such as 1% BSA, 1% sucrose in 1X PBS) and incubate for 1 hour.
4. Repeat washes as in step 2.
5. Prepare several dilutions of antigen to be detected, add 100 µL/well of each dilution to replicate wells and incubate for 1 hour.
6. Repeat washes as in step 2.
7. Dilute detection antibody (HRP conjugated) to desired concentration in an assay buffer (such as 0.2% BSA, 0.2% Tween-20), add 100 µL/well, and incubate for 1 hour on a shaker platform.
8. Repeat washes as in step 2.
9. Mix Lumi-Phos HRP reagent solutions A and B in 1:1 ratio (see section on product handling instructions) and add 50 to 100 µL/well.
10. Read the plate on a luminometer.

Troubleshooting Tips for ELISA

There are mainly two types of problems associated with the detection of antigens in an ELISA.

1. **Signal problems:** Very high, weak and no signal
2. **Background problems:** High non-specific background

Signal Problems	Possible Causes	Troubleshooting Tips
1. Weak signal	• Low concentrations of capture and HRP detection antibodies • Poor antigen – antibody binding • Poor binding of capture antibody to the well surface • Inhibition of antigen-antibody binding or of HRP enzyme by components in wash and blocking buffers • Expired or contaminated Lumi-Phos HRP substrate	• Use higher concentrations of capture and HRP detection antibodies • Use highly specific antibodies • Use plates with high binding capacity • Check the components in blocking and wash buffers and replace them with new buffers • Use a new lot of substrate
2. Very high signal	• High concentrations of antigen and antibodies	• Titrate antigen and antibodies
3. High signal followed by fast signal decay	• Very high concentration of HRP antibody	• Use more diluted HRP antibody
4. No signal	• Lack of antigen-antibody binding • Inactive HRP enzyme • Inhibition of antigen-antibody binding or of HRP enzyme by components in wash and blocking buffers • Expired substrate	• Replace with highly specific antibodies • Use a new lot of HRP detection antibody • Check the components in blocking and wash buffers and replace them with new buffers • Use a new lot of substrate

Background Problems	Possible Causes	Troubleshooting Tips
1. Very high background in wells with no antigen	• Use of high amount of HRP antibody • Insufficient washing of the wells • Inadequate blocking	• Use more diluted HRP detection antibody • Increase number of washes • Block longer period of time or change blocking agent
2. Higher than normal Lumi-Phos HRP substrate background	• Contaminated substrate • Vigorous mixing of A and B solutions • Using the A and B mix immediately after mixing • Light exposure • Exposure to metal ions	• Use a new lot of substrate • Mix A and B by inverting the container • Use after 5 minutes of mixing A and B • Avoid exposure to light • Avoid exposure to metal ions

Suggested Product Handling Instructions

Storage:

Store bottled product at 2-8º C.

Protect from exposure to direct light.

Do not freeze.

Use:

***Use in subdued light. Indirect incandescent lighting is preferred. Exposure to direct light will cause elevated background.**

1) Allow substrate solutions A and B to equilibrate to room temperature (Approximately 1 hour for 100 mL of product).
2) Gently invert (4-5 times) solutions A and B in their packaged containers to assure homogeneity prior to dispensing. **Avoid vigorous agitation of reagent.**
3) Dispense the needed amount of solutions A and B into two separate new opaque HDPE or PP plastic containers. Containers should be opaque or covered with aluminum foil to protect from direct light (daylight or artificial).
4) Mix equal parts of solutions A and B in a new opaque HDPE or PP plastic container.
5) Allow mixed working solution to sit for at least 2 minutes prior to use.
6) **Do not mix again before use.**
7) For ELISA applications, aliquot the working solution into assay wells, while protecting from light contamination, and read on a luminometer.

Repackaging:

Repackaging of Lumi-Phos HRP reagent is discouraged as reliability can be compromised by contamination. Bottling is available from Lumigen to suit the volume demands for your specific work process.

If you choose to repackage, new opaque HDPE or PP plastic containers are required. **Reusing and washing containers can lead to contamination and subsequent high background.**