Lumi-Phos HRP (PS-atto)

Chemiluminescent Reagent

Product Application Instructions

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). 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.

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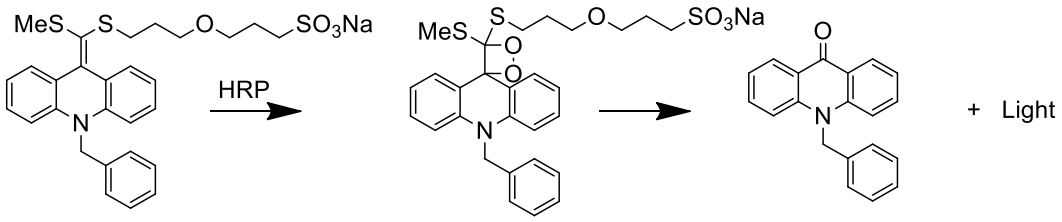
22900 W 8 Mile Road • Southfield MI 48033 • USA
Telephone: **248 351-5600** • Toll Free: **877 LUMIGEN**
Facsimile: **248 351-0518**

Product Overview

Lumi-Phos HRP reagent is a chemiluminescent substrate for the detection of HRP conjugated antibodies in ELISA. Reaction of the substrate with an HRP label rapidly generates sustained high-intensity luminescence for maximum detection sensitivity in ELISA assays.

Product Structure and Generation of Chemiluminescent Signal

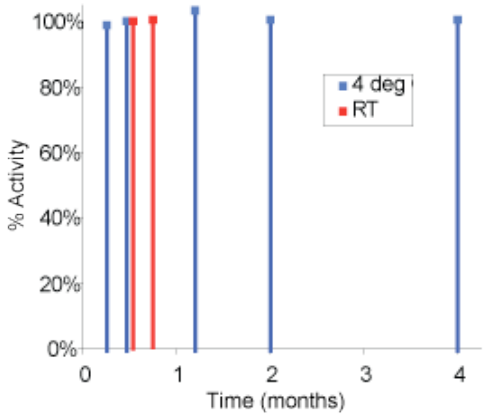


Product Characteristics and Applications

- Very rapid onset of chemiluminescence; stable peak intensity reached in seconds.
- Excellent sensitivity - less than 10^{-21} 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.
- Sustained luminescence - timing is less critical.
- Light intensities can be read at any time to produce linear calibration curves.

Stable Working Solution

An important and unique feature of Lumi-Phos HRP reagent is its extended storage stability. The working solution prepared by 1:1 mixing of aqueous solutions A and B is stable at room temperature for at least one week and at 2-8°C for at least one month. Lumi-Phos HRP is the only peroxidase substrate that permits the working solution to be stored and used on luminometer with one injector.



Storage Stability of Lumi-Phos HRP reagent

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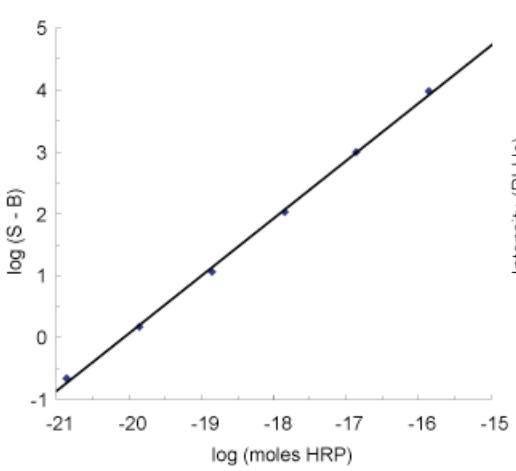
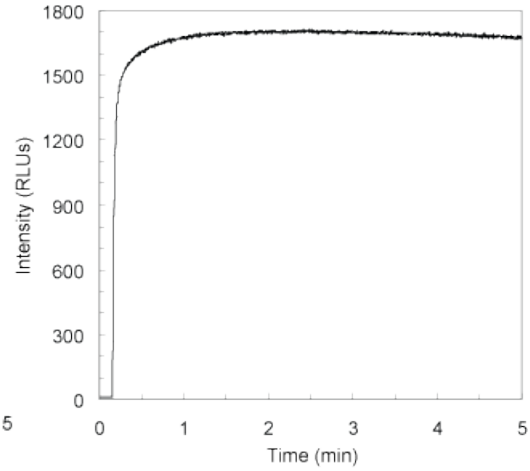


Log plot of net signal and moles of HRP detected with Lumi-Phos HRP reagent



Solution kinetics of Lumi-Phos HRP reagent signal intensity over a 5 minute period

An example of ELISA data generated using Lumi-Phos HRP reagent

Chemiluminescent ELISA Detection of Thyroid Stimulating Hormone (TSH) with Lumi-Phos HRP reagent					
Capture antibody - Mouse anti-human TSH @ 7.5µg/mL;					
Detection antibody - Goat anti-human TSH @ 0.01µg/mL					
TSH Sample Conc. µIU/mL		**Rep 1**	**Rep 2**	**Mean**	**S/S0**
S4	**10**	3341	3438	3389	416
S3	**4**	1490	1602	1546	210
S2	**0.5**	210	225	218	30
S1	**0.1**	44	51	47	6.5
S0	**0**	7.3	7.4	7.35	1

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Product Handling Instructions

Storage:

Store bottled product at 2 – 8ºC.
Protect from light contamination.
Do not freeze.

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

1) Allow solutions A and B to come to room temperature (Approximately 1 hour for 100 mL)
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 amount needed of solutions A and B into separate containers. Containers should be opaque or covered with aluminum foil to protect from direct light (daylight or artificial).
4) For working solution, mix solutions A and B in the ratio of 1:1 in a new container and let stand for 5 minutes.
5) In subdued light, add the working solution to microtiter wells with HRP bound protein or nucleic acids.
6) Read chemiluminescent signal 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. The high temperatures in the container molding process destroy most or all potential contaminants.
Reusing and washing of containers can lead to contamination and subsequent high background.

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. For consistent results, perform the antibody incubation and washing steps at ambient temperature (22-25ºC).

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. Let the microtiter plate stand for 2 minutes and read on a plate 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	• Use a new lot of substrate • Mix A and B by inverting the container • Use after 5 minutes of mixing A and B

Lumigen Product Matrix

	Lumigen ECL Plus	Lumigen ECL Extra	Lumigen ECL Ultra	Lumi-Phos Plus	Lumi-Phos 530	Lumigen APS-5	Lumi-Phos HRP	Lumigen SPARCL	Lumigen HyPerBlu
Application	Western Blotting	Western Blotting	Western Blotting	Southern Northern Blotting	ELISA	ELISA	ELISA	ELISA, Other Proximity Assays	H_2O_2 Detection
Enzyme	HRP	HRP	HRP	AP	AP	AP	HRP	HRP	Direct, Non-enzymatic
Sensitivity	Low pg to mid fg	Low pg to mid fg	Mid to low fg	Single Copy Gene Detection	Low pg to fg	Low pg to fg	Low pg to fg	Low pg	2.6 nM
Signal Duration	Up to 5 Hours	Up to 6 Hours	Up to 8 Hours	Up to 24 Hours	Up to 24 Hours	Up to 4 Hours	Up to 5 Hours	Instantaneous Flash	Up to 8 Hours
Shelf Life	2 Years	1 Year	1 Year	2 Years	2 Years	1 Year	1 Year	1 Year	2 Years
Storage Conditions	2 - 8° C	2 - 8° C	2 - 8° C	2 - 8° C	2 - 8° C	2 - 8° C	2 - 8° C	2 - 8° C	2 - 8° C

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