Lumigen APS-5

Chemiluminescent Reagent

Product Application Instructions

Catalog Number **AP5-101**

Contents: **100 mL single ready-to-use formulation**

Description **Lumigen APS-5** is recommended primarily for chemiluminescent solution assays and for ELISA detection of proteins bound with antibodies conjugated with alkaline phosphatase (AP). However, it can also be used for the detection of proteins on Western blots with AP- conjugated detection antibodies.

Note: Lumigen APS-5 is invented, developed and manufactured by Lumigen.

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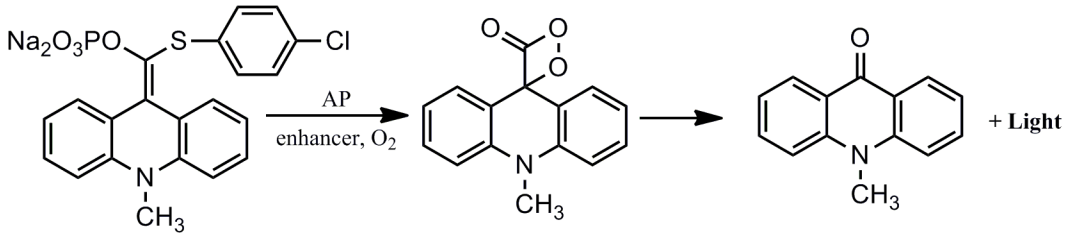
LUMIGEN
22900 W 8 Mile Road • Southfield MI 48033 • USA
Telephone: **248 351-5600** • Toll Free: **877 LUMIGEN**
Facsimile: **248 351-0518**

Product Overview

Lumigen APS-5 is a chemiluminescent substrate for the detection of any AP-conjugated molecules such as antibodies in ELISA assays. Reaction of the substrate with an AP label rapidly generates sustained high intensity luminescence for maximum detection sensitivity.

Product Structure and Generation of Chemiluminescent Signal



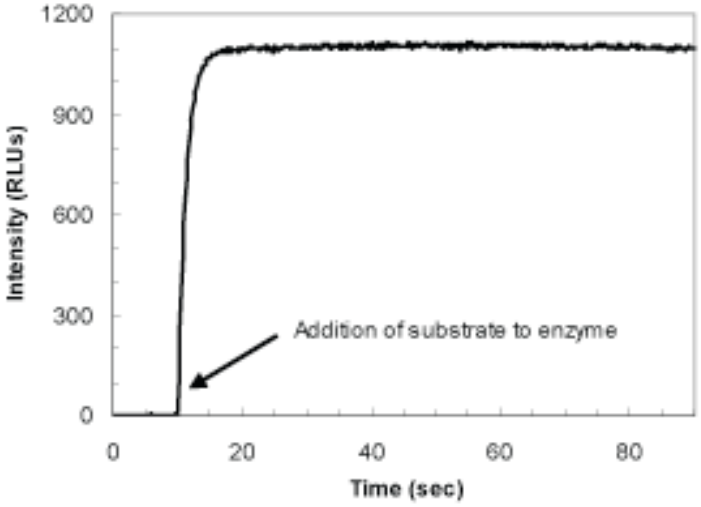
Product Characteristics and Applications

Lumigen APS-5 utilizes a unique technology for the chemiluminescent detection of alkaline phosphatase conjugates and provides superior sensitivity and ease of use. Reaction of the acridan substrate with AP produces rapid and sustained high-intensity chemiluminescence. Lumigen APS-5 is ideally suited for measuring AP activity in solution assays and for detecting antigens with AP-linked antibodies in immunoassays.

Homogeneous Solution Assay:

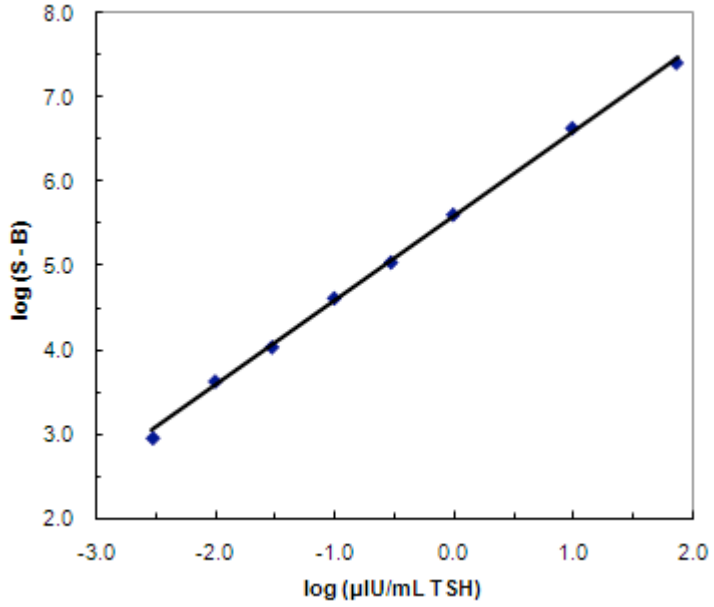
Some of the important features of Lumigen APS-5 are:

- Excellent sensitivity - less than 10^{-19} moles of AP can be measured.
- Rapid development of peak signal reduces assay time and increases throughput.
- Linear calibration curves with slopes of log-log plots equal to 1.0 -- one order of magnitude more enzyme yields one order of magnitude more luminescence.
- Sustained luminescence -- timing is less critical. Light intensities can be read at any time to produce linear calibration curves
- Analytical results are insensitive to temperature from 22°C to 35°C reducing the need for precise temperature control.



Rapid Chemiluminescent Immunoassay:

A sandwich immunoassay for thyroid stimulating hormone (TSH) performed with an Immulite TSH Third Generation TSH Assay kit (Siemens) yielded a linear plot of 0.003 mIU/L to 75 mIU/L with excellent assay precision. Lumigen APS-5 was substituted for the normal dioxetane substrate, Lumigen PPD in the Immulite assay.



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Product Handling Instructions

Storage:

Store bottled product at 2-8º C.
Protect from light contamination.
Do not freeze.

Use:

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

1) Allow the substrate solution to come to room temperature (Approximately 1 hour for 100 mL of product).
2) Gently invert (4-5 times) solution in its packaged container to assure homogeneity prior to dispensing.
3) Dispense the amount needed of solution into new opaque HDPE or PP plastic container. Containers should be opaque or covered with aluminum foil to protect from direct light (daylight or artificial).
4) Aliquot the solution into assay wells while protecting from light contamination.
5) Store unused product in original container at 2-8º C for up to 1 year.

Repackaging:

Repackaging of Lumigen products 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 potential contaminants.
Reusing and washing of containers can lead to contamination and subsequent high background.

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ELISA Assay – Equipment and Material Required

- White or black high protein binding microtiter plates
- Capture antibody
- Antigen and reference set of antigen concentrations
- Detection antibody AP-conjugate
- Blocking solution such as 1% BSA
- Washing buffers such as 1X TBS with 0.05% Tween-20
- Lumigen APS-5 reagent
- Microplate luminometer

Important Notes and Precautions

It is essential that the capture antibody and AP 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 for chemiluminescent detection, the antibody stocks may be diluted and used in the range of 10µg/mL to1µg/mL for capture antibody and from 0.01µg/mL to 0.001µg/mL for AP-conjugated 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

1. Coat white or black microtiter wells such as FluoroNunc Maxisorp with 100 µL/well of capture antibody (10 to 1µg/mL in 1X TBS) by incubating for 30 – 60 min. on a shaker platform at ambient temperature.
2. Wash 3X with 300µL/well of 1X TBST (with 0.05% Tween-20).
3. Add 300µL/well of blocking agent (such as 1% BSA, 1% sucrose in 1X TBS) and incubate for 1 hour at 37°C.
4. Repeat washes as in step 2.
5. Prepare several dilutions of antigen to be detected along with reference antigen with known concentration and add 100µL/well of each dilution and of the reference to replicate wells. Incubate for 1 hour at room temperature on a shaker platform.
6. Repeat washes as in step 2.

7. Dilute detection antibody (AP-conjugated) to desired concentration in an assay buffer (such as 0.2% BSA, 0.2% Tween-20), add 100µL/well, and incubate for 1 hour at ambient temperature on shaking platform.
8. Repeat washes as in step 2.
9. Add APS-5 to wells (50 to 100µL/well).
10. Immediately 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 AP detection antibodies	• Use higher concentrations of capture and AP detection antibodies
	• Poor antigen – antibody binding	• Use highly specific antibodies
	• Poor binding of capture antibody to the well surface	• Use plates with high binding capacity
	• Inhibition of antigen-antibody binding or of AP enzyme by components in wash and blocking buffers	• Check the components in blocking and wash buffers and replace them with new buffers
	• Expired or contaminated APS-5 substrate	• 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 AP antibody	• Use higher dilution of AP antibody
4. No signal	• Lack of antigen-antibody binding	• Replace with highly specific antibodies
	• Inactive AP enzyme	• Use a new lot of AP detection antibody
	• Inhibition of antigen-antibody binding or of AP enzyme by components in wash and blocking buffers	• Check the components in blocking and wash buffers and replace them with new buffers
	• Expired substrate	• 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 AP antibody • Insufficient washing of the wells • Inadequate blocking	• Use higher dilution of AP detection antibody • Increase number of washes • Block longer period of time or change blocking agent
2. Higher than normal APS-5 substrate background	• Contaminated substrate	• Use a new lot of substrate

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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