Lumigen APS-5

Chemiluminescent Reagent

Product Overview

Catalog Number **AP5-101, AP5-1000**

Contents: **100 mL or 1 L single ready-to-use formulation**

Description

Lumigen APS-5 is a proprietary acridan based chemiluminescent substrate for ELISA detection of alkaline phosphatase (AP) conjugated molecules. It utilizes a unique technology for the chemiluminescent detection of alkaline phosphatase conjugates which combines excellent sensitivity with ease of use. Reaction of the acridan substrate with an AP label rapidly produces high intensity chemiluminescence for sensitive detection. Lumigen APS-5 is ideally suited for solution assays of phosphatase activity and for phosphatase-linked immunoassays.

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

Applications:

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 10 µg/mL to 1 µg/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. Maintaining temperature during antibody incubation may help to achieve consistent results.

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 1 µg/mL in 1X TBS) by incubating for 30 – 60 min. on a shaker platform at ambient temperature.
2. Wash 3X with 300 µL/well of 1X TBST (with 0.05% Tween-20).
3. Add 300 µL/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 100 µL/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 100 µL/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 100 µL/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 • Poor antigen – antibody binding • Poor binding of capture antibody to the well surface • Inhibition of antigen-antibody binding or of AP enzyme by components in wash and blocking buffers • Expired or contaminated APS-5 substrate	• Use higher concentrations of capture and AP 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 AP antibody	• Use higher dilution of AP antibody
4. No signal	• Lack of antigen-antibody binding • Inactive AP enzyme • Inhibition of antigen-antibody binding or of AP enzyme by components in wash and blocking buffers • Expired substrate	• Replace with highly specific antibodies • Use a new lot of AP 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 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 • Light exposure • Exposure to metal ions	• Use a new lot of substrate • 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 the substrate solution to equilibrate 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 needed amount 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.
Reusing and washing of containers can lead to contamination and subsequent high background.

Data Sheet Lumigen: APS-5 AP Substrate

Homogeneous Solution Assay:

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

- Rapid development of peak signal reduces assay time and increases throughput.
- Linear calibration curves of log-log plots show one order of magnitude more enzyme yields one order of magnitude more luminescence.

Rapid Chemiluminescent Immunoassay:

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

Linear Calibration Curve

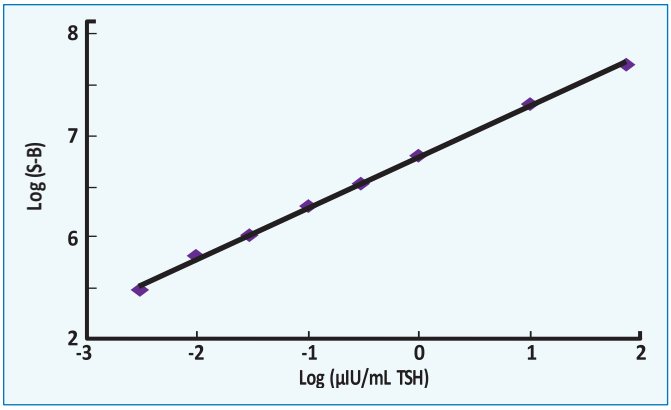

Figure 1. A sandwich immunoassay for thyroid stimulating hormone (TSH) performed on Immulite Immunoassay System

Rapid Peak Intensity

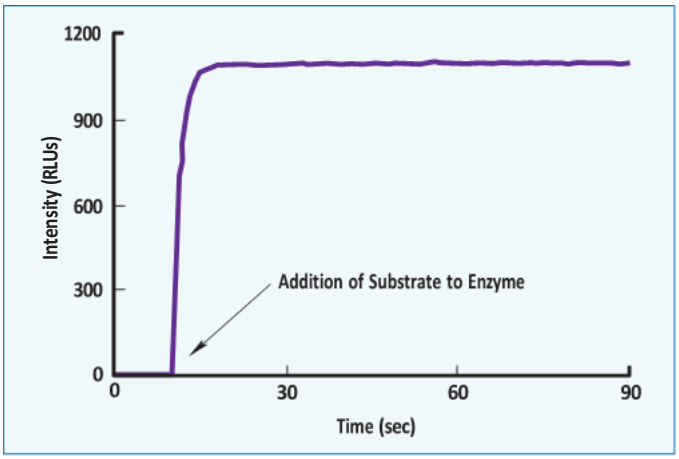

Figure 2. Lumigen APS-5 reaches a sustained maximum light-output peak intensity within seconds of substrate addition

Product Specifications

Enzyme	Alkaline Phosphatase (AP)
Application	ELISA

Ordering Information

Description	Catalog Number
Lumigen APS-5 (100 mL)	AP5-101
Lumigen APS-5 (1 L)	AP5-1000