Lumigen® HyPerBlu

Chemiluminescent Reagent

Product Application Instructions

Catalog Number **A97286**

Contents **5 mL single ready-to-use formulation**

Description **Lumigen HyPerBlu** is used for direct chemiluminescent detection of hydrogen peroxide without involvement of any peroxidase enzyme. It can also be used for one-step indirect detection and quantification of any oxidase enzyme activity or their substrates that generate hydrogen peroxide in the reaction.

Note: Lumigen HyPerBlu is invented, developed and manufactured by Lumigen.

Table of Contents **Page**

LUMIGEN

22900 W 8 Mile Road • Southfield MI 48033 • USA

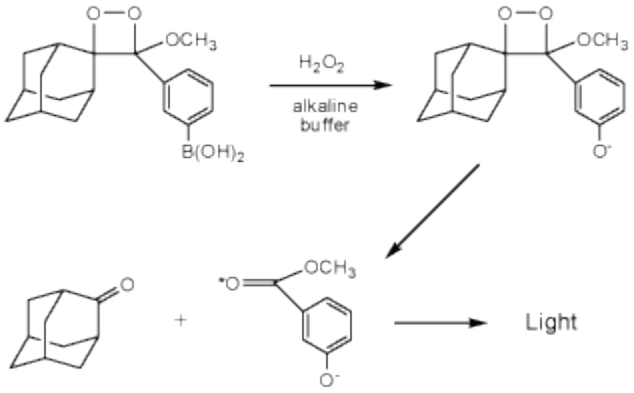
Telephone: **248 351-5600** • Toll Free: **877 LUMIGEN**

Facsimile: **248 351-0518**

Product Overview

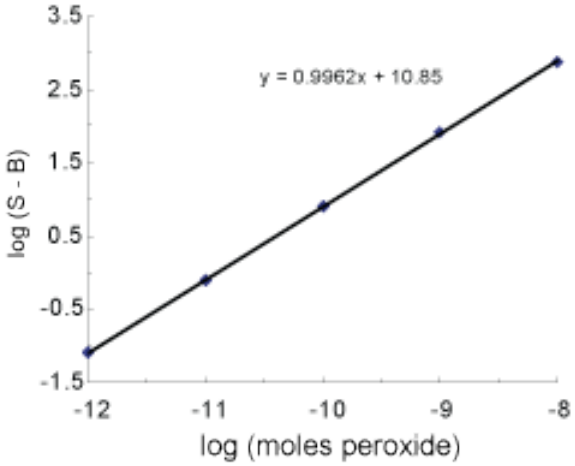
Lumigen HyPerBlu is a novel chemiluminescent substrate that directly reacts with hydrogen peroxide (without peroxidase) generating a sustained high-intensity chemiluminescence. HyPerBlu can be used to detect hydrogen peroxide that is released in any of the multitude of oxidase reactions and thus useful in indirectly quantifying oxidases and their substrates.

Product Structure and Generation of Chemiluminescent Signal

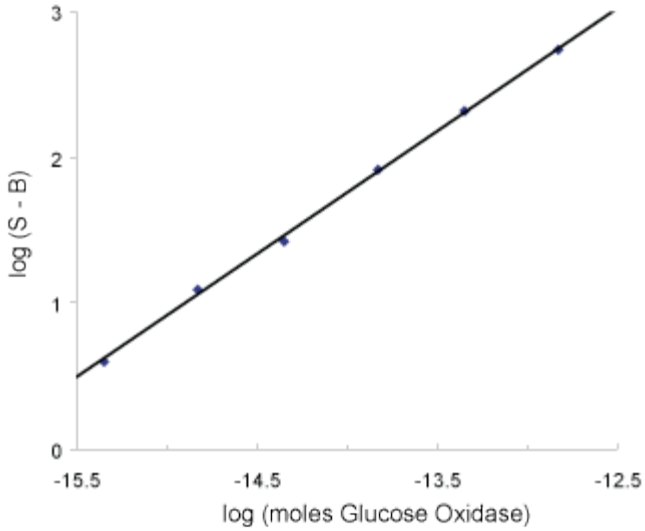


Product Characteristics and Applications

- Excellent sensitivity in solution assays
- Broad dynamic range with bright sustained chemiluminescence
- Single ready-to-use reagent formulation for convenience



In addition to the direct non-enzymatic detection of hydrogen peroxide, Lumigen HyPerBlu can also be used for one-step indirect detection of oxidase enzymes or their substrates that produce hydrogen peroxide. In the example shown below, HyPerBlu was used to quantify glucose oxidase activity. Similarly, the glucose substrate of this enzyme can be quantified in a reaction with fixed amount of glucose oxidase.



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

Oxidase Reactions

Some of the other oxidase reactions where Lumigen HyPerBlu can be used to quantify the enzymes and substrates include:

Dopamine + O_2 + H_2O → 3, 4-dihydroxyphenylacetaldehyde + NH_3. + **H_2O_2**
(**Monoamine oxidase A**)

Hypoxanthine + H_2O + O_2 ⇌ Xanthine + **H_2O_2**
(**Xanthine oxidase**)

Uric acid → Allantoin + CO_2 + **H_2O_2**
(**Uricase**)

D-Galactose + O_2 ⇌ D- Galacteraldehyde + **H_2O_2**
(**Galactose oxidase**)

Cholesterol + O_2 → 4-Cholesten-3-one + **H_2O_2**
(**Cholesterol oxidase**)

Acetylcholine + H_2O → Choline + Acetate
(**Acetylcholine esterase**)

Choline + H_2O + O_2 → Betaine + **H_2O_2**
(**Choline oxidase**)

L-Glutamate + O_2 + H_2O → α-Ketoglutarate + NH_3. + **H_2O_2**
(**Glutamate oxidase**)

Product Handling Instructions

Storage:

Store bottled product at 2-8ºC.
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 solution to come to room temperature (Approximately 1 hour for 100 mL of product).
2) Gently invert (4-5 times) solution in its packaged containers to assure homogeneity prior to dispensing.
3) Dispense the amount needed of solution into a 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 2-8ºC for up to 2 years.

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.**

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

Lumigen HyPerBlu Assay – Equipment and Material Required

- White microtiter plates – 96 or 384 wells
- PMT luminometer or CCD Imager
- Urea peroxide reference standard
- Oxidase enzyme
- Lumigen HyPerBlu reagent

Protocol Steps:

1. Prepare microtiter plate wells by adding 5 uL reactions of oxidase and its substrate.
2. Incubate to generate hydrogen peroxide for certain period of time depending on the oxidase characteristics.
3. Aliquot 5 uL of urea peroxide standard dilutions to empty wells on the plate.
4. Add 5 uL of Lumigen HyPerBlu to all the wells.
5. Incubate 15 minutes at room temperature.
6. Read the wells on either PMT luminometer or CCD imaging system with appropriate integration times.

Troubleshooting Tips for Lumigen HyPerBlu Assay

There are mainly two types of problems associated with the detection of hydrogen peroxide.

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 hydrogen peroxide • Low pH • Insufficient incubation time. • Expired or contaminated HyPerBlu • Competition for hydrogen peroxide by other substrates	• Increase concentration of oxidase • Adjust to pH 8-10 • Increase incubation time • Use a new lot of substrate • Confirm that no other hydrogen peroxide consuming substrates are present (e.g. HRP)
2. Very high signal	• High concentrations of hydrogen peroxide	• Titrate oxidase or reduce oxidase incubation time

Signal Problems	Possible Causes	Troubleshooting Tips
3. No signal	• Insufficient hydrogen peroxide	• Increase oxidase concentration
	• Incompatible pH	• Adjust pH of assay to pH 8-10
	• Detection equipment failure	• Check the detection equipment to verify it is in a luminescent mode and not filtering any wavelength of light.
	• Expired substrate	• Use a new lot of substrate
	• Hydrogen Peroxide consumed by natural process	• Confirm that hydrogen peroxide is the endpoint for the biological process being studied

Background Problems	Possible Causes	Troubleshooting Tips
1. Very high background in wells	• Contaminated detection equipment	• Clean detection unit according to manufacturer's instructions
	• Elevated temperature	• Confirm HyPerBlu is at room temperature
	• Equipment contamination by hydrogen peroxide.	• Examine equipment usage, and cleaning procedures

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

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