LUMIGEN
A Beckman Coulter Company

Lumigen HyPerBlu

Chemiluminescent Reagent for Direct Hydrogen Peroxide Detection

Lumigen HyPerBlu™ Chemiluminescent Reagent is a novel ready-to-use substrate for the direct detection of hydrogen peroxide. Reaction of the substrate with hydrogen peroxide rapidly generates sustained high-intensity luminescence for maximum detection sensitivity in solution assays. Coupled with oxidases the Lumigen HyPerBlu substrate also allows one step indirect quantitation of oxidase substrates or the oxidase itself.

Lumigen HyPerBlu substrate is suitable for automation and miniaturization to 384-well or 1536-well format. Lumigen HyPerBlu reagents wide tolerance for common assay additives (eg DMSO), lack of intermediaries, chemical specificity, one-component structure and excellent stability lead to assay simplicity, excellent robustness and unmatched convenience for high throughput screening laboratories.

- **Direct detection of peroxide, oxidase enzymes or their substrates**
- **Single ready-to-use reagent formulation with long stability for convenience**
- **Broad dynamic range with bright sustained chemiluminescence**
- **Excellent sensitivity in solution assays**

Lumigen HyPerBlu Technology

Lumigen HyPerBlu reagent is a novel chemiluminescent substrate that directly reacts with hydrogen peroxide (without peroxidase) rapidly generating a sustained high-intensity chemiluminescence. The signal intensity can be measured on PMT based plate reading luminometers or CCD imaging systems.

High-throughput screening (HTS) assays present a variety of unique challenges for assay technologies. Naturally fluorescing compounds, biological intermediaries and target labels can decrease the robustness of an assay. Lumigen HyPerBlu reagent demonstrates a variety of advantages over traditional fluorescent peroxide measurement. Long stability of the formulation combined with the wide hydrogen peroxide dynamic range (>5 orders) allow for more sensitive and reproducible assays. By directly measuring the peroxide rather than relying on enzymatic intermediates the assay is also less susceptible to interference.

Reaction Mechanism

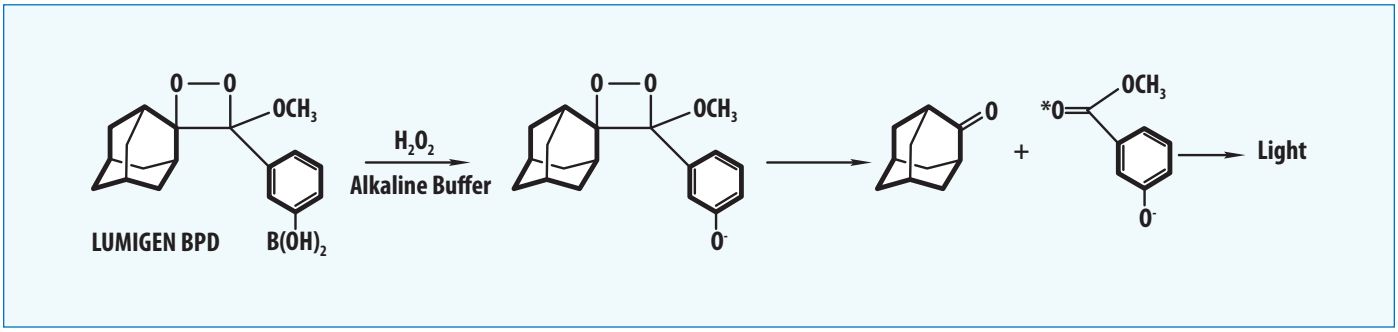

Figure 1. Reaction Mechanism of Lumigen HyPerBlu Reagent
Lumigen HyPerBlu assays utilize a technology based on the specific reaction of a dioxetaneboronic acid with hydrogen peroxide.

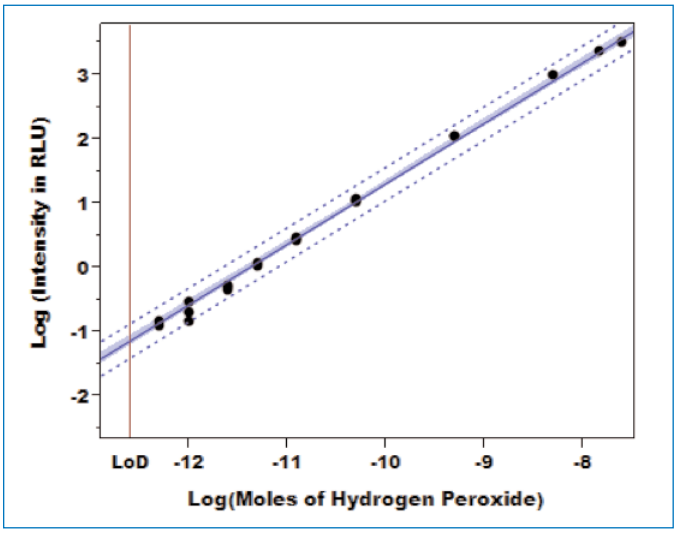

Figure 2. Hydrogen Peroxide Sensitivity - Linear Detection Over 5 Orders of Magnitude

3 replicates of 50 µL serial dilution of Urea Peroxide were prepared and added to a white 96 well plate. 50 µL of Lumigen HyPerBlu reagent was added to each well. Readings reported at the 24 minute time point.

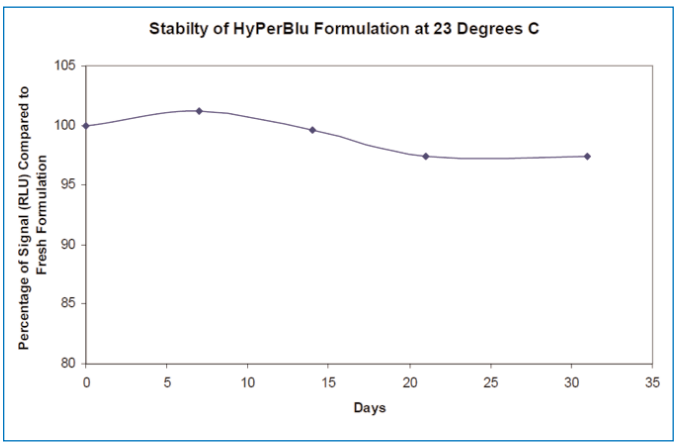

Figure 3. Stability of Reagent

Lumigen HyPerBlu reagent was stored at room temperature (~23°C). Samples were measured against a freshly prepared formulation weekly and were still producing over 95% of the signal of the new lot after 31 days.

Indirect Detection of Oxidases

The measurement of hydrogen peroxide produced enzymatically is of growing interest for the study of a variety of biological functions and the analysis of physiological effects of oxidative stress. Lumigen HyPerBlu chemiluminescent reagent offers a no-label enzyme-free technique for the detection of oxidase activity. Because the Lumigen HyPerBlu substrate directly measures hydrogen peroxide produced from oxidative reactions, assays are significantly simpler, more flexible, and avoid complications associated with label-based technologies.

Lumigen HyPerBlu assays can be used for the one step detection of oxidases or oxidase substrates that produce free hydrogen peroxide. If an excess of substrate is present, the chemiluminescent signal will be proportional to the amount of enzyme. Conversely, if a fixed amount of enzyme is present and the substrate is varied, the substrate quantity can be assayed.

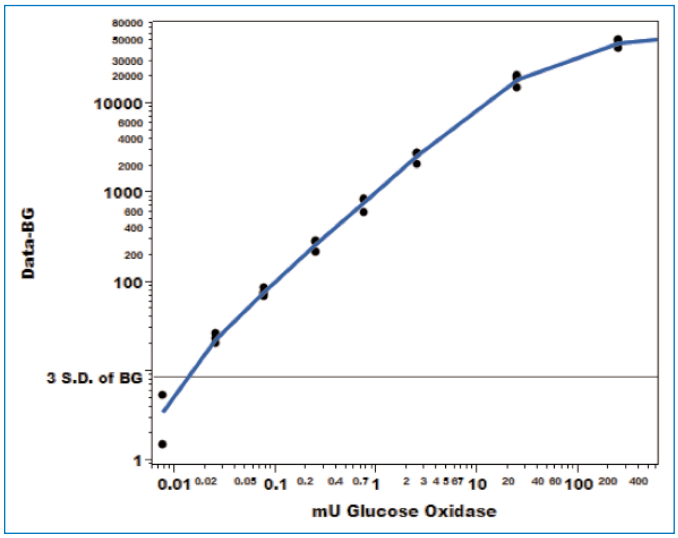

Figure 4. One Step Detection of Glucose Oxidase

4 replicates of Glucose Oxidase standard concentrations were prepared by serial dilution. Glucose was then added to each well. After 30 minutes of incubation at room temperature Lumigen HyPerBlu reagent was added. Readings at the 24 minute time point are reported.

Product Specifications

Detection	Hydrogen Peroxide (directly) Glucose or Glucose Oxidase (indirectly)
Sensitivity	2.6 nM Hydrogen Peroxide
Signal Duration	Up To 8 Hours
Storage Conditions	2 - 8°C; Store in amber bottle to protect from light
Shelf Life	2 years
Working Solution	1 part, ready-to-use formulation

Ordering Information

Description	Catalog Number
Lumigen HyPerBlu (5 mL)	**HPB-00005**
Lumigen HyPerBlu (100 mL)	**HPB-100**
Lumigen HyPerBlu (1 L)	**HPB-1000**

Please visit **www.LUMIGEN.com** or contact LUMIGEN to request a quote.

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