

# Lumigen HyPerBlu provides sensitive chemiluminescent detection of hydrogen peroxide



## Beckman Coulter

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Read the complete protocol online:

[www.biotechniques.com/protocols/113571](http://www.biotechniques.com/protocols/113571)

Lumigen HyPerBlu reagent is a one solution chemical substrate based on dioxetane chemiluminescent technology. With HyPerBlu reagent, a direct reaction with hydrogen peroxide produces an intense sustained luminescent signal making it an excellent choice for examination of oxidase activity. The signal intensity is sufficient for measurement on PMT based plate reading luminometers or CCD imaging systems. HyPerBlu technology detection is suitable for automation and miniaturization to 384-well or 1536-well format. HyPerBlu reagent's wide tolerance for common assay additives (e.g., DMSO), lack of intermediaries, chemical specificity, one-component structure, and excellent stability lead to assay simplicity and excellent robustness.

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 technology avoids these challenges utilizing a technology based on the specific reaction of a dioxetaneboronic acid with hydrogen peroxide. The resulting production of light is based on the half-life of the intermediate compound. Traditional assay methodologies for the measurement of hydrogen peroxide use horseradish peroxidase (HRP) to catalyze oxidation of colorimetric or fluorescent compounds. These drawbacks limit their stability and reproducibility.

## Materials

- PMT luminometer (Labsystems)
- 384-well white microtiter plates (Generic)
- Urea peroxide standard
- Oxidase enzyme
- Lumigen HyPerBlu Chemiluminescent Reagent

## Protocols

1. Prepare well with oxidase and analog as appropriate for chosen study.
2. Add 5  $\mu$ L standard or sample to each well.
3. Incubate per oxidase characteristics.
4. Add 5  $\mu$ L Lumigen HyPerBlu Chemiluminescent Reagent.
5. Incubate 20–30 min at room temperature.

6. Detect with 1-s integration per well for PMT luminometer systems, or 5 s per plate for CCD imaging systems.

## Data Analysis

Lower detection limits (LDL) and dynamic range for Lumigen HyPerBlu was determined by preparing a dilution series of urea peroxide standards. The signal obtained was plotted against the standard concentrations tested in the assay. The LDL was determined by using the background mean + 3  $\times$  SD.

## Glucose Oxidase Test Assay

Glucose was oxidized with glucose oxidase in aqueous solution and the peroxide generated was assayed with Lumigen HyPerBlu (Figure 2). Solutions containing  $1.5 \times 10^{-13}$  to  $4.5 \times 10^{-16}$  moles of glucose oxidase were analyzed in triplicate, in a one-step format. Wells were incubated at 25°C with a solution of 0.1 M glucose. Chemiluminescence intensity was measured at 22 min.

The glucose oxidase assay shows a linear response over the tested range (Figure 3). This same method could be used in a study of enzymatic response to various conditions or in a detection test for glucose itself.

## Results and discussion

As shown in Figure 1, an LDL of  $5 \times 10^{-13}$  moles of Hydrogen Peroxide was achieved. The wide linear dynamic range of 5 logs gives this assay great flexibility to measure a variety of processes with minimal process scaling.

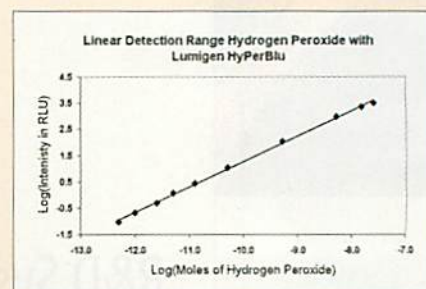


Figure 1. Lumigen HyPerBlu Chemiluminescent Reagent demonstrates a linear detection range from  $2.5 \times 10^{-8}$  to  $5 \times 10^{-13}$  moles hydrogen peroxide.

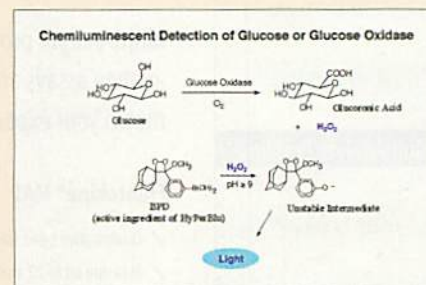


Figure 2. Reaction schematic of Hydrogen Peroxide detection with Lumigen HyPerBlu Chemiluminescent Reagent.

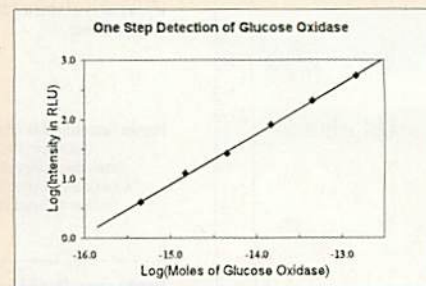


Figure 3. Detection study of glucose oxidase using Lumigen HyPerBlu Chemiluminescent Reagent.

Stability of performance of Lumigen HyPerBlu when stored at 2–8°C was shown to have a variation of <5% in one year. When Lumigen HyPerBlu is kept at 23°C, the product stability shows a variation of <3% in 31 days. This ease of use can be compared favorably with traditional peroxide detection technologies that require biological detection components.

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