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HyPerBlu

Superior High Throughput Glucose Oxidase Activity Assay with Lumigen HyPerBlu Chemiluminescent Reagent

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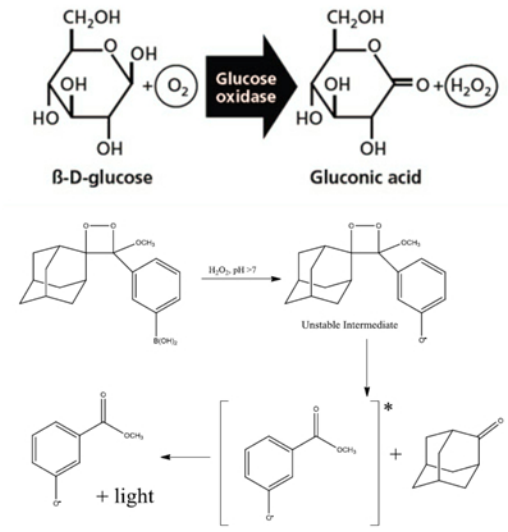
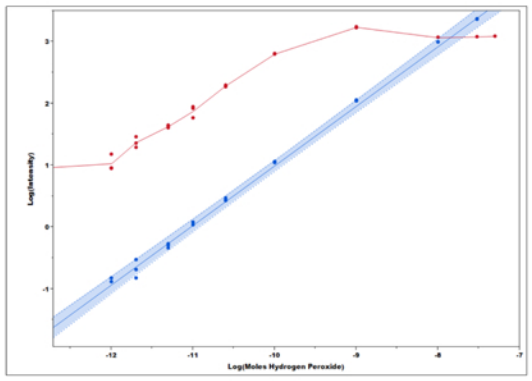
Abstract

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. Innovative, robust techniques are required for demanding high-throughput environments. Lumigen HyPerBlu reagent is a new substrate that offers a variety of advantages in this area. Lumigen HyPerBlu chemiluminescent reagent offers a no-label enzyme-free technique for the detection of oxidase activity. Because Lumigen HyPerBlu reagent directly measures hydrogen peroxide produced from oxidative reactions, assays with this new substrate are significantly simpler, more flexible and avoid complications associated with label-based technologies. Compared to traditional absorbance and fluorescent assays Lumigen HyPerBlu assays offer a wide dynamic range, improved sensitivity, and excellent stability. Glucose oxidase is an ideal candidate to compare detection technologies; it is well understood, commonly used in research applications by itself and coupled with other targets, and has significant biological relevance. Solutions containing 0.03 mU to 300 mU of glucose oxidase were measured and a linear response curve was obtained. Conversely, solutions containing 0.1 µM to 10 mM solutions of glucose were shown to also generate a linear response. Comparisons were completed with a resorufin-based fluorescent probe (Amplex Red).

Background



HyPerBlu – Hydrogen Peroxide Sensitivity



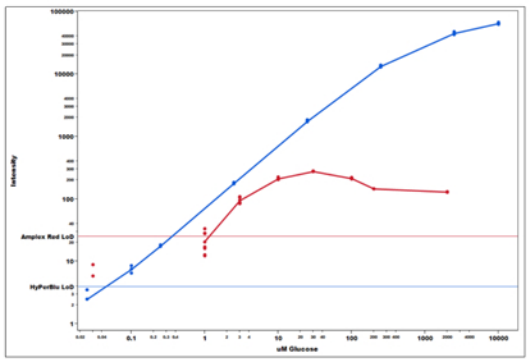
Three replicates of 50 µL serial dilution of Urea Peroxide (Aldrich) were prepared in deionized water and added to a white 96 well plate. 50 µL of Lumigen HyPerBlu reagent were added to each well. The intensity was measured using a Lumistar Optima (BMG). 50 µL of Amplex Red (Life Technologies) were tested with a second set of replicates on a Flouroscan Ascent (Labsystems). Readings at the 24 minute time point are reported.

Glucose Assay – Amplex Red

Materials and experimental details were sourced from the Amplex Red Glucose/Glucose Oxidase Assay Kit (A22189 Life Technologies). Eight replicates of 50 µL of Glucose standard concentrations were prepared by serial dilution in 0.25 M phosphate buffer pH 7.4 and added to a 96-well plate. A detection solution was prepared with 100 µM Amplex Red, 0.2 U/mL HRP and 2 U/mL glucose oxidase in 0.25 M phosphate buffer pH 7.4. 50 µL of the detection reagent was added to the standard. After 30 minutes of incubation at room temperature the intensity was measured using 530 nm excitation and 590 nm emission on a Flouroscan Ascent (Labsystems).

Glucose Assay – HyPerBlu

Glucose and Glucose Oxidase were sourced from the Amplex Red Glucose/Glucose Oxidase Assay Kit (A22189 Life Technologies). Four replicates of 50 µL of Glucose standard concentrations were prepared by serial dilution in 0.25 M phosphate buffer pH 7.4 and added to a 96-well plate. 50 µL of 5 U/mL Glucose Oxidase were added to each well. After 30 minutes of incubation at room temperature 100 µL of Lumigen HyPerBlu reagent was added to each well. The intensity was measured using a Lumistar Optima (BMG). Readings at the 24 minute timepoint are reported.



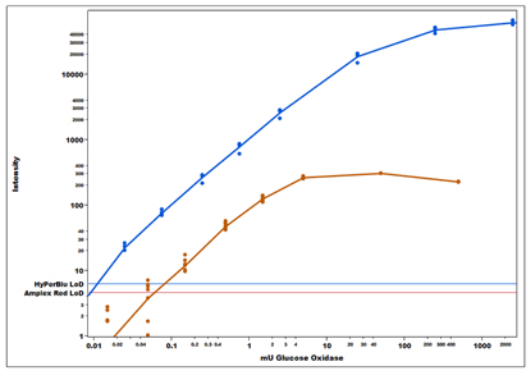
Limit of Detection was calculated as 3 standard deviations above the background.

Glucose Oxidase Assay – Amplex Red

Materials and experimental details were sourced from the Amplex Red Glucose/Glucose Oxidase Assay Kit (A22189 Life Technologies). Eight replicates of 50 µL of Glucose standard concentrations were prepared by serial dilution in 0.25 M phosphate buffer pH 7.4 and added to a 96-well plate. A detection solution was prepared with 100 µM Amplex Red, 0.2 U/mL HRP and 100 mM Glucose in 0.25 M phosphate buffer pH 7.4. 50 µL of the detection reagent was added to the standard. After 30 minutes of incubation at room temperature the intensity was measured using 530 nm excitation and 590 nm emission on a Flouroscan Ascent (Labsystems).

Glucose Oxidase Assay – HyPerBlu

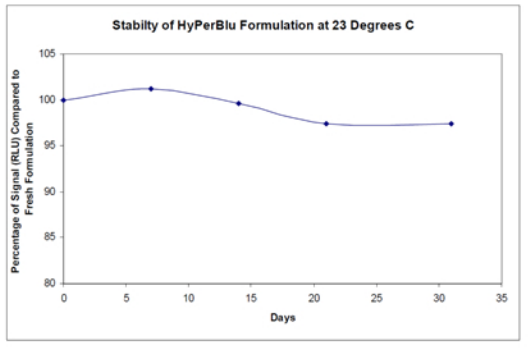
Glucose and Glucose Oxidase were sourced from the Amplex Red Glucose/Glucose Oxidase Assay Kit (A22189 Life Technologies). Four replicates of 50 µL of Glucose Oxidase standard concentrations were prepared by serial dilution in 0.25 M phosphate buffer pH 7.4 and added to a 96-well plate. 50 µL of 450 mM Glucose were added to each well. After 30 minutes of incubation at room temperature 100 µL of Lumigen HyPerBlu reagent was added to each well. The intensity was measured using a Lumistar Optima (BMG). Readings at the 24 minute timepoint are reported.



Limit of Detection was calculated as 3 standard deviations above the background.

HyPerBlu Stability

A sample of HyPerBlu reagent was stored at room temperature (~23 °C). Samples were measured against a freshly prepared formulation. After 31 days the reagent produced over 95% of the performance of the new lot. This stability extends to 2 years when stored at 2-8 °C. By comparison Amplex Red components are stored at -20 °C and are highly unstable when combined, the manufacturer (Life Technologies) recommends that they be used immediately.



Conclusion

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 less susceptible to interference. Amplex Red's manufacturer also warns against air, light, and recommends a restrictive pH of only 7-8. (Lumigen HyPerBlu reagent can be used at pH 7-12).

In the case of Glucose Oxidase the Lumigen HyPerBlu formulation exploits a wide dynamic range, using higher glucose levels than Amplex Red, to achieve a range two orders of magnitude wider. The Amplex Red actually produces less fluorescence at high levels of peroxide compared to moderate levels increasing assay design difficulties and decreasing flexibility. These advantages as well as the very reproducible low background gives the Lumigen HyPerBlu Glucose assay advantages in sensitivity as well as dynamic range.