

# LUX Biotechnology GLOWELL™ luminometry standards on the POLARstar OPTIMA



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- Light output from Glowell was similar across experiments, indicating uniform light readings by POLARstar OPTIMA
- Glowell standards allowed relative light units to be converted to quantitative measurements
- Whilst biological controls varied, Glowell standards were consistent in their light output.

## Introduction

Glowell™ devices are certified calibration reference materials. They provide calibration and validation of luminescence and fluorescence measuring equipment, whilst simultaneously acting as standards for experimental data. Each Glowell emits a pre-measured, stable light output, generated by a chemical light source. The aim of this experiment was to use a Glowell to determine (i) the stability of the light measured by the POLARstar OPTIMA, between experiments carried out on different days and (ii) the consistency of biological standards between experiments.

## Materials and Methods

- Glowell™ 96-Well Microplate Standards (Green Light) (LUX biotechnology, # GLO-001)
- Methanol (Fisher Scientific)
- Crude Gaussia luciferase 45% (LUX Biotechnology # NL-GAU)
- Free base coelenterazine (LUX Biotechnology # NF-CTZ)
- Distilled water
- POLARstar OPTIMA, with luminescence optics, BMG LABTECH.
- Microplates, white 96 well, Nunc.

3 Glowell standards, each with a different light intensity spanning 3 orders of magnitude in total, were placed into wells of a white 96-well microplate. Four other wells were filled with 200 µL of 2.5 µg/mL Gaussia luciferase, dissolved in water and containing 10 µM free base coelenterazine. Light output from each well was measured for 0.1 s, every 10 s for 500 s. This was repeated a day later, using the same Glowell standards but with freshly made solutions.

NB. 1 mg coelenterazine was dissolved in 500 µL methanol, before dilution to a stock concentration of 200 µM with standard distilled water. 10 µL of this was added to each well (containing 190 µL of luciferase) immediately before luminometry.

POLARstar OPTIMA parameters:

- Read Mode: Plate
- Gain: 3275
- Positioning delay: 0.2 s
- No. kinetic windows: 1
- Number of cycles: 50
- Measurement start time: 0 s
- Measurement interval time 0.1 s
- Cycle time: 10 s

Shake before first cycle for 2 seconds, double orbital (3 mm), to mix well contents.

Data obtained was evaluated using the BMG LABTECH FLUOstar Excel™ evaluation package.

## Results and Discussion

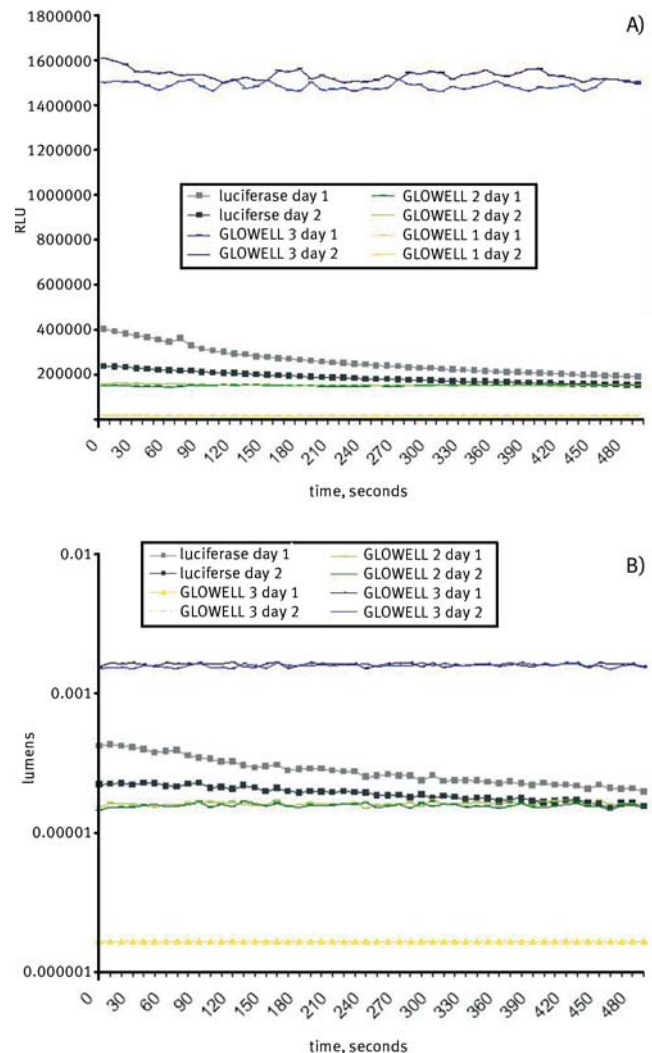
Glowells produce a stable light output of known intensity (measured according to National Physics Laboratory Standards). This allows the conversion of RLU to quantitative units (lumens).

Conversion, to Glowell 3:

$$\text{Absolute light output} = \frac{\text{Sample RLU}}{\text{Glowell 3 RLU}} \times \text{Glowell 3 lumens}$$

Glowell 3 is 1.63712E-06 lumens

This conversion was applied to the data, Figure 1.



**Fig. 1:** The results of an investigation of how emission intensity changes over time between two separate experiments ('day 1' and 'day 2'). A) The RLU obtained from Glowells and luciferase samples at each timepoint (Luciferase data is the average reading from four separate wells). B) RLU at each timepoint were converted to quantitative measurements (lumens) using Glowell 3.

There were overall differences in light output at each timepoint between day 1 and 2 (Figure 1). The difference in light output at each time point between day 1 and 2, was determined and averaged, Table 1.

**Table 1:** The % differences in RLU from Glowwell standards and luciferase controls, between day 1 and day 2. The % difference in RLU at each timepoint from day 1 and 2 was determined and the mean calculated.

	GLOWELL 1 (RLU)	GLOWELL 2 (RLU)	GLOWELL 3 (RLU)	Luciferase control (RLU)
Average % difference between day 1 and day 2	4.7	1.7	3.12	40.27

Glowwell standards are designed to emit stable light output and as expected, little variation was observed in the light detected from each Glowwell standard (Table 1). This indicated the POLARstar OPTIMA provided reliable and uniform readings between experiments.

In contrast, luciferase activity was lower in the second experiment, compared to the first. Luciferase controls, are often included in experiments to provide a base-line with which to compare test samples. However, as illustrated here, these, and other biological controls are subject to variation (Table 1). The inclusion of a Glowwell in an

experiment ensures variation is due to sample variation rather than machine variation and provides a means of comparing light output between experiments. Glowwell devices (Figure 2) can also be used to calibrate readings from different machines, and this is of particular interest for high throughput screening applications over extended time periods.

In summary, the inclusion of a Glowwell allowed the reliability of the POLARstar OPTIMA to be ascertained. Unlike biological controls, Glowwell standards provide a long term, stable light output. Finally, Glowwell devices produce a pre-measured light output allowing conversion of relative light units to quantitative measurements.



**Fig. 2:** Left: Glowwell calibration devices  
Right: Glowwell calibration devices in a 96-well plate

Glowwell devices are available in a number of models, colours and light intensities. For more information please visit the LUX biotech website at [www.luxbiotech.com](http://www.luxbiotech.com) or contact us at [info@luxbiotech.com](mailto:info@luxbiotech.com)

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