

Dual Luciferase Reporter (DLR) Assay Certification on the Omega Series of Readers

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Introduction

The Dual-Luciferase® Reporter Assay or DLR is widely used to study gene transcription and regulation. The DLR assay is a two step reaction that uses two luciferase enzymes, Firefly and *Renilla* (Figure 1). The Firefly reaction is initiated, followed by its quenching and the subsequent initiation of the *Renilla* reaction. The dual measurement of these two enzymes allows for an experimental measurement and a transfection control measurement to be done at the same time. This dual reporting of each sample allows a quantitative result based on the normalization of the *Renilla* luciferase (transfection control). More information on the DLR assay and its certification requirements are available on Promega's website at www.promega.com and in the technical manual¹.

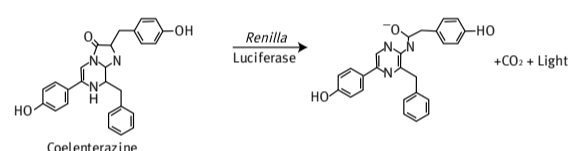
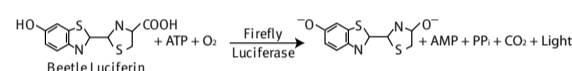


Fig. 1: Firefly and *Renilla* luminescent reactions

The certification process consists of 3 parts: Quenching, consistency and tubing adsorption. The quenching experiment indicates that the Firefly luciferase has been fully quenched with the addition of the Stop and Glo® reagent. The consistency experiment indicates that the instrument can maintain a relative standard deviation of less than 5% (%CV). The tubing adsorption experiment indicates whether the tubing used in the injectors will affect the outcome of the DLR assay over time. These 3 experiments were conducted on the POLARstar Omega, which achieved DLReady™ certification. Luminescence detection and injection is identical for the POLARstar Omega, FLUOstar Omega, and LUMIstar Omega, making all 3 readers DLReady™ certified.

Assay Design

The dual luciferase assay is a fast reaction with 2 injection steps, one for the Firefly substrate (Luciferase Assay Reagent II or LAR II) and one for the Stop and Glo® buffer which contains the Firefly quencher and the *Renilla* substrate (Figures 2).



Fig. 2: Dual Luciferase Reaction - Luciferase Assay Reagent II (LAR II) is injected in the first step and the Firefly reaction is started. Stop and Glo® buffer is injected in the second step, which quenches the Firefly reaction and initiates the *Renilla* reaction.

The reaction requires an injection and a measurement for 12 seconds (to quantitate the Firefly luminescence) and then another injection and another 12 second measurement (to quantitate the *Renilla* luciferase).

Materials and Methods

- BMG LABTECH POLARstar Omega, Ser # 415-027
- White, flat-bottom 96-well Costar® plates (Catalog # 3912)
- Promega's DLR certification kit
- Recombinant Firefly and *Renilla* luciferase provided by Promega

Instrument settings

Read Mode:	Well mode
Optics:	3 mm Luminescence
Positioning Delay:	0.2 sec
Measurement start time:	0.0 sec
No. of intervals:	48
Interval time:	0.50 sec
Emission filter:	empty
Injection speed:	230 µl/sec
Injection start time:	0 and 12 sec

These experiments were performed as described in the Promega Instrumentation Certification documentation¹. Each test varies slightly from running the kit as a whole. Each of the 3 criteria for certification were run according to Promega's guidelines.

For data calculation, the relative luminescence units are summed over two ranges:

Range 1 -
Firefly luminescence
(cycles 7-25 or 3.0-12 secs).

Range 2 -
Renilla luminescence
(cycles 30-48 or 14.5-23.5 secs).

Results and Discussion

Criterion 1: Quenching of >10,000 Firefly/*Renilla*
Recombinant Firefly luciferase (3.05 ng/ml) shows an average quenching of ~100,000 fold (n=24) on the POLARstar Omega (Figure 3). Divide the background subtracted Firefly luminescence by the background subtracted *Renilla* (no *Renilla* was used here).

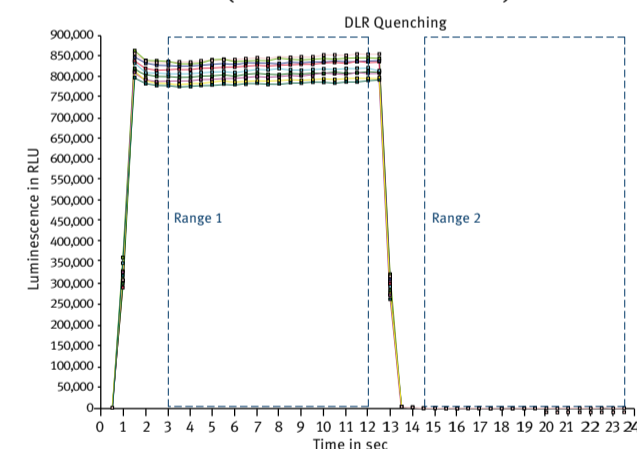


Fig. 3: Criterion 1 - Graph from the MARS evaluation software for Firefly luciferase showing quenching (~100,000 fold) (n=24)

Criterion 2: Consistency showing <5% CV
In the first part, 15x Firefly to *Renilla* luciferase concentration was used and in the second part, 30x *Renilla* to Firefly luciferase concentration was used. For the first part, Firefly luminescence showed a 2.3% CV and *Renilla* luminescence a 2.5% CV (Figure 4). For the second part, Firefly luminescence showed a 2.2% CV and *Renilla* luminescence a 2.0% CV (Figure 5).

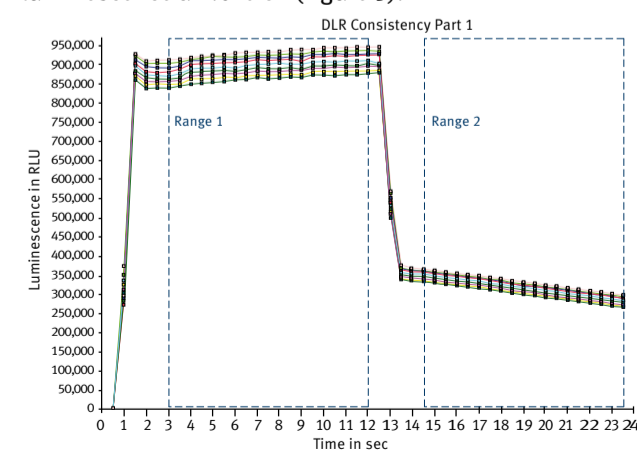


Fig. 4: Criterion 2 - Graph from the MARS evaluation software showing part 1 of consistency (%CV=2.3 for Firefly, %CV=2.5 for *Renilla*, n=24)

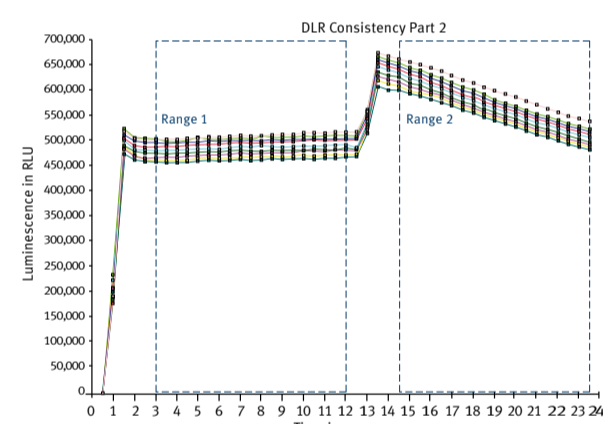


Fig. 5: Criterion 2 - Graph from the MARS evaluation software showing part 2 of consistency (%CV=2.2 for Firefly, %CV=2.0 for *Renilla*, n=24)

Criterion 3: Tubing Adsorption showing <5% CV after 10 minutes

For this reaction, 15x Firefly to *Renilla* luciferase concentration was used.

Twelve replicates were run and then twelve more were run after 10 minutes to test for possible adsorption by the tubing. The values and their %CVs are well within the criterion (Table 1).

Table 1: Criterion 3 - Tubing Adsorption shows little change after 10 minutes

	Firefly Luciferase Average (n=12)	%CV
RLU	1.055 x 10 ⁷	2.5
RLU (10 min)	1.030 x 10 ⁷	1.9

	<i>Renilla</i> Luciferase Average (n=12)	%CV
RLU	3.711 x 10 ⁶	1.8
RLU (10 min)	3.793 x 10 ⁶	2.5

Conclusion

The Omega series of microplate readers from BMG LABTECH has been granted DLReady™ certification based on the results published in this application note.

These results were obtained with the POLARstar Omega microplate reader and can be extended to the FLUOstar and the LUMIstar Omegas.



Fig. 6: BMG LABTECH's microplate reader LUMIstar Omega

The FLUOstar Omega is the first multifunctional microplate reader that has a high speed, UV/Vis spectrometer for absorbance, as well as having fluorescence and luminescence capabilities.

The POLARstar Omega is a step above the FLUOstar, having simultaneous dual emission detection for fluorescent polarization assays.

The LUMIstar Omega (Figure 6) is a dedicated luminescence reader that is fully upgradeable to the FLUOstar or POLARstar Omegas. The Omega is the third series of life science readers from BMG LABTECH that have been DLReady™ certified by Promega.

Certification has been granted to the Galaxy and OPTIMA series of plate readers, as well as the NOVOstar plate reader with micropipettor.

References

1. Promega, Corp. Dual Luciferase Reporter Assay Technical Manual (TM046) (8/06).
2. DLR and the DLReady logo are trademarks of Promega Corporation

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