Dual Luciferase Reporter (DLR) Assay Certification on the CLARIOstar® using LVF monochromators™

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The Microplate Reader Company
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- The CLARIOstar has been validated to perform Promega's DLR assay
- Detection of Firefly and *Renilla* luciferase signals automated using two onboard reagent injectors

Introduction

The DLR or Dual-Luciferase Reporter Assay has become the go-to assay for use in studies of regulation of gene transcription. Two enzymes are used in DLR, Firefly and *Renilla* luciferase (Figure 1). These enzymes are employed in a two-step reaction. Consecutive measurement of the Firefly and *Renilla* luciferase signals is performed with an intervening quenching that stops the reaction of the Firefly luciferase.

Dual measurement allows for one enzyme signal to serve as the experimental measurement (Firefly) while the second enzyme will serve as the transfection control (*Renilla*). A quantitative result is obtained from the measurement of both enzymes in a single sample. This quantitation is based on the normalization to the Renilla luciferase signal. Additional information about the DLR assay and certification requirements can be found in the DLR technical manual[1] and Promega's website (www.promega.com)

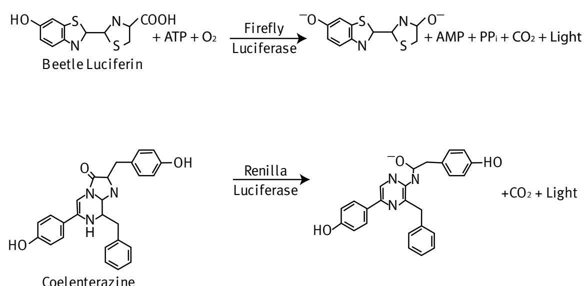

Fig. 1: ***Bioluminescent reactions of Firefly and Renilla***

The Certification Process

In order to attain DLR certification three criteria must be fulfilled:
The first criterion is the quenching experiment. This will indicate whether the Stop and Glo® reagent, added in injection step 2, has successfully quenched the Firefly luciferase reaction initiated by Luciferase Assay Reagent II which was added in injection step 1.
The second criterion is the consistency experiment. These experiments determine whether a relative standard deviation of less than 5% (%CV) can be maintained by the instrument at two different concentrations of Firefly and *Renilla* luciferase.
The tubing adsorption experiment is the third criterion. This experiment shows whether over time the tubing used in the instruments injectors will have an effect on the DLR assay.

These experiments were carried out on the CLARIOstar (Figure 2).

Fig. 2: ***CLARIOstar*** *- BMG LABTECH's multifunctional microplate reader with advanced LVF monochromators™, filters and spectrometer.*

Assay Design

Dual luciferase assays use fast reactions and employ 2 injection steps. The Luciferase Assay Reagent II (LAR II) which contains the Firefly substrate is delivered by the first injection. A Firefly reaction quencher and the *Renilla* substrate are contained in the Stop and Glo® buffer which is delivered by the second injection. Two 12 second reactions are sequentially performed to quantitate Firefly luminescence followed by *Renilla* luminescence (Figure 3).

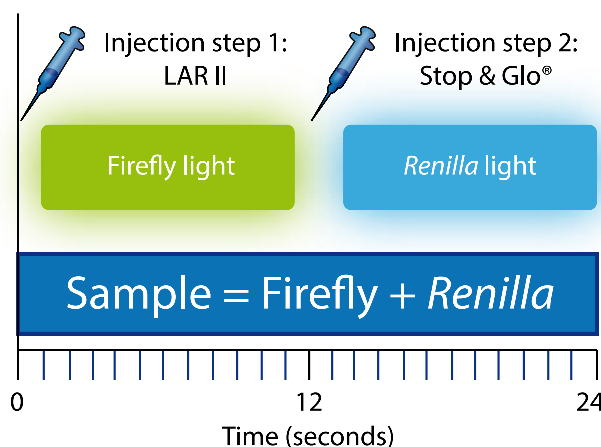

Fig. 3: ***Dual Luciferase Reaction*** *Luciferase Assay Reagent II (LAR II) is injected in step 1 to initiate the Firefly luciferase reaction. In the second step the injection of Stop and Glo® step quenches the Firefly reaction while starting the reaction for Renilla Luciferase.*

Materials and Methods

- ☐ White, flat bottom 96-well Corning plates (3917)
- ☐ Promega's DLR certification kit (E1960)
- ☐ Recombinant Firefly and *Renilla* luciferase provided by Promega

The CLARIOstar was set up with the following parameters:

Read Mode:	Well Mode
Optics:	Monochromator (520-620 nm)
Positioning Delay:	0.2 sec
Measurement start times:	2.5 and 14.5 sec
No. of intervals:	18 and 19
Interval time:	0.50 sec
Injection speed	220 µl/sec
Injection start times:	0 and 12 sec
Gain:	3600
Focal Height:	10.0 mm
Optic used:	Top

Experiments were performed in accordance with the Promega instructions. Although each of the tests were performed consistent with Promega guidelines, each experiment varies slightly from running the kit as a whole.

The relative luminescence units were summed over two ranges for data calculation:
Range 1: 2.5 – 11 secs or cycles 1 – 18 (Firefly luminescence)
Range 2: 15 – 23.5 secs or cycles 20 – 37 (*Renilla* luminescence)

Results and Discussion

Criterion 1: Quenching of >10,000 Firefly/Renilla

Recombinant firefly luciferase (3 ng/mL) exhibited quenching on the CLARIOstar that was >10,000 fold (DLR requirements). This was calculated by dividing blank corrected Firefly luminescence by blank corrected *Renilla* luminescence (no *Renilla* was used in this experiment).

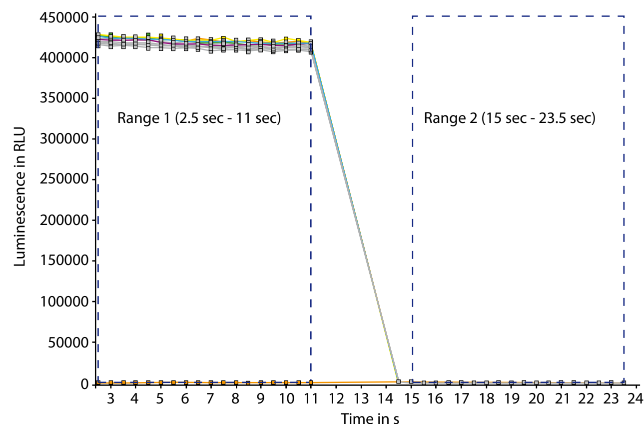

Fig. 4: ***Criterion 1***- *Firefly luciferase quenching (>10,000 fold [n=34]) Graph taken from MARS evaluation software*

Criterion 2: Consistency showing < 5% CV

For criterion 2, a 15 X Firefly to *Renilla* luciferase concentration was used for part 1, while a concentration of 30 X *Renilla* to Firefly was used in part 2. In part 1, the CV's for Firefly luminescence and *Renilla* luminescence were 2.0% and 1.5% respectively. In part 2; Firefly luminescence exhibited a 0.5% CV and *Renilla* luminescence exhibited a 2.3% CV (Figure 5).

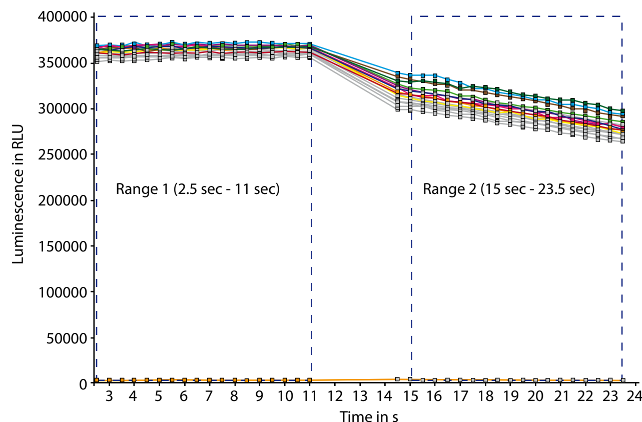

Fig. 5: ***Criterion 2***- *Data from MARS data evaluation software shows part 2 of consistency experiment graphically (CV's for Firefly and Renilla = 0.5% and 2.3% respectively [n=48])*

Criterion 3: Tubing Adsorption showing <5% CV after 10 minutes. For this test 15 X Firefly to *Renilla* was used. Twenty-four replicates were run followed by twenty-four more replicates after a 10 minute wait to test for possible tubing adsorption. The % CVs are all 2 or lower; and thus solidly within the criterion (Table 1).

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

	Firefly Average (%CV) [n=12]	***Renilla* Average (%CV) [n=12]**
RLU	7.006 E+6 (0.7)	1.768 E+5 (1.4)
RLU (10 min)	6.910 E+6 (0.5)	1.762 E+5 (2.0)

Conclusion

The CLARIOstar microplate reader has been granted DLReady™ certification based on the results published in this application note. The CLARIOstar joins previous instruments from BMG LABTECH which have attained DLReady certification by Promega. The Galaxy, Optima and Omega series of readers have all been previously certified. Certification has also been granted to the NOVOstar plate reader with micropipettor and most recently to the PHERAstar *FS*.

References

1. Promega, Corp. Dual Luciferase Reporter Assay System Technical Manual (TM 040) (6/11)
2. DLR and the DLReady logo are trademarks of Promega Corporation

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