Dual Luciferase Reporter (DLR) Assay Certification on the PHERAstar *FS*

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- The PHERAstar *FS* has been certified as ready to perform the DLR assay from Promega
- Instantaneous detection of Firefly and *Renilla* luciferase is possible due to simultaneous measurement and two onboard reagent injectors

Introduction

To study the regulation of gene transcription, the DLR or Dual-Luciferase Reporter Assay is commonly used. The two luciferase enzymes used in DLR, Firefly and *Renilla* are employed in a two-step reaction (Figure 1). The Firefly and *Renilla* luciferase signals are measured consecutively with intervening quenching of the Firefly luciferase.

Using dual measurements allow one enzyme signal to serve as the experimental measurement (Firefly) while the second enzyme serves as a cell viability / transfection control (*Renilla*). The measurement of both enzymes in a single sample yields a quantitative result based on the normalization to the *Renilla*. Please refer to the DLR technical manual[1] and Promega's website (www.promega.com) for additional information about the DLR assay and certification requirements.

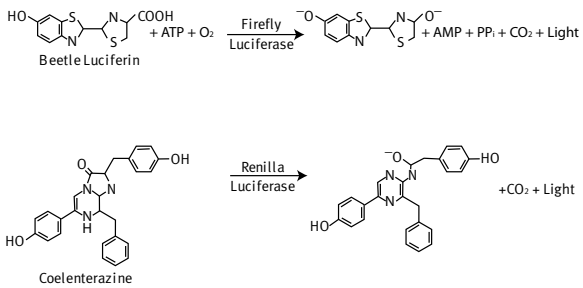

Fig. 1: ***Bioluminescent reactions of Firefly and Renilla***

The Certification Process

Three criteria must be fulfilled in order to attain DLR certification.

The first criterion indicates whether the second injection step, which contains the Stop and Glo® reagent, has completely quenched the Firefly luciferase reaction. This is called the quenching experiment. To pass the second criterion or consistency experiment a relative standard deviation of less than 5% (%CV) must be maintained by the instrument at two different concentrations of Firefly and *Renilla*. Finally, the third criterion is the tubing adsorption experiment which indicates the effect the tubing used in the instruments injectors will have on the DLR assay over time. This effect should be minimal.

In order to attain DLReady™ certification; experiments were performed using the PHERAstar *FS* (Figure 2)

Fig. 2: ***The PHERAstar FS-*** *BMG LABTECH's high performance, multimode reader for HTS*

Assay Design

Two injection steps are employed by the dual luciferase assay to carry out the fast reactions. In the first injection the Firefly substrate is delivered in the Luciferase Assay Reagent II (LAR II). The second injection consists of the Stop and Glo® buffer which delivers a Firefly reaction quencher and the *Renilla* substrate. To quantitate Firefly luminescence followed by *Renilla* luminescence two sequential 12 second reactions are performed (Figure 3).

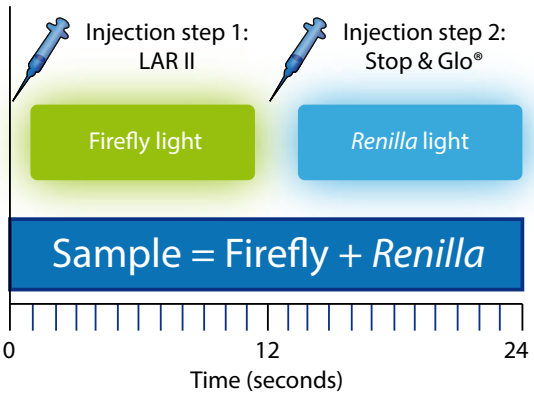

Fig. 3: ***Dual Luciferase Reactions*** *In the first step, to initiate the Firefly luciferase reaction, Luciferase Assay Reagent II (LAR II) is injected. The injection of Stop and Glo® in the second step starts the Renilla Luciferase reaction while quenching the Firefly.*

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 PHERAstar *FS* was set up with the following parameters:

Read Mode:	Well Mode
Optic Module:	LUM plus
Positioning Delay:	0.2 sec
Measurement start time:	0.0 sec
No. of intervals:	48
Interval time:	0.50 sec
Injection speed	220 µl/sec
Injection start times:	0 and 12 sec
Gain:	3600
Focal Height:	15.0 mm
Optic used:	Top
Aperture spoon:	none

Promega documentation, instructions and guidelines were followed for the performance of each of these experiments. While each of the criteria conform to these guidelines there is slight variation from running the kit in its entirety.

Summing of the relative luminescence units over two ranges was performed for data calculation:
Range 1: 2.5 – 11.5 secs or cycles 6 – 24 (Firefly luminescence)
Range 2: 14.5 – 23.5 secs or cycles 30 – 48 (*Renilla* luminescence)

Results and Discussion

Criterion 1: Quenching of ›10,000 Firefly/*Renilla*

The quenching seen on the PHERAstar *FS* for recombinant Firefly luciferase (0.3 ng/mL) was greater than the 10,000 fold required for DLReady™ status. In order to calculate this; Firefly luminescence was divided by *Renilla* luminescence (no *Renilla* was used).

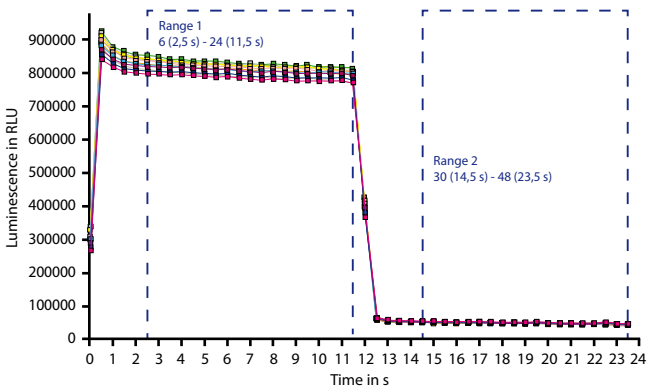

Fig. 4: ***Criterion 1-*** *Graph showing Firefly luciferase quenching taken from MARS evaluation software (›10,000 fold [n=24])*

Criterion 2: Consistency showing ‹ 5% CV

For part 1 of criterion 2, 15 X Firefly to *Renilla* luciferase concentration was used, while part 2 used 30 X *Renilla* to Firefly. The CV for Firefly luminescence was 1.52% and *Renilla* luminescence exhibited a 1.76% CV in part 1. Furthermore, Firefly luminescence exhibited a 1.58% CV and the CV for *Renilla* was 0.56% in part 2 (Figure 5).

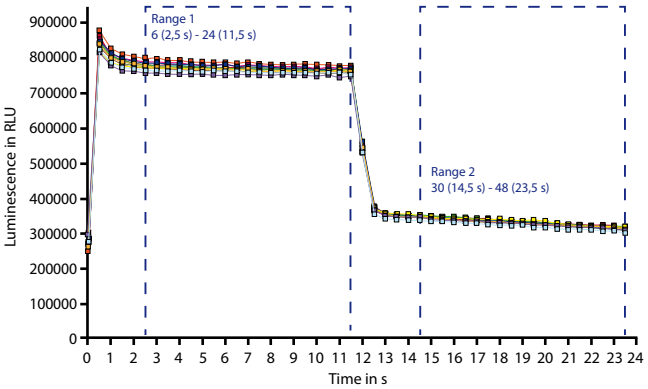

Fig. 5: ***Criterion 2-*** *Part 2 of consistency is shown graphically with data from MARS data evaluation software (CV's for Firefly and Renilla = 1.58% and 0.56% respectively [n=24])*

Criterion 3: Tubing Adsorption show ‹ 5% CV after 10 minutes

Similar to criterion 2 part one; 15 X Firefly to *Renilla* was used for this test. Twelve replicates were run followed by twelve more replicates with an intervening 10 minute wait to test for possible tubing adsorption. As with the other tests the % CVs are less than 2 and therefore clearly 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	1.552 E+7 (1.5)	8.925 E+5 (1.7)
RLU (10 min)	1.536 E+7 (1.1)	9.546 E+5 (1.8)

Conclusion

Based on the results published in this application note, the PHERAstar *FS* microplate reader has been granted DLReady™ certification. The PHERAstar *FS* is a multifunctional reader suitable for HTS applications. Other instruments from BMG LABTECH which have previously attained DLReady™ certification by Promega include; the Galaxy, Optima and Omega series of readers. The NOVOstar plate reader with micropipettor and the CLARIOstar, which features an Advanced Monochromator are also certified DLReady™.

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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