Dual Luciferase Reporter (DLR) assay certification

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- **Promega´s DLR assay had been validated on the Omega, CLARIOstar® and PHERAstar® *FS* microplate readers from BMG LABTECH**

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 manual.

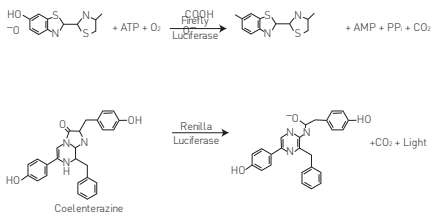

Fig. 1: Bioluminescent reactions of Firefly and *Renilla*.

The certification process consists of 3 parts: Quenching, consistency and tubing adsorption.

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 3 experiments were conducted on the FLUOstar® and LUMIstar® Omega as well as on the CLARIOstar and PHERAstar *FS* which achieved DLReady™ certification.

Assay Principle

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 (Figure 2).

Fig. 2: Dual Luciferase Reaction - Luciferase Assay Reagant 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 & Methods

- White, flat-bottom 96-well Costar® plates
- Promega's DLR certification kit
- Recombinant Firefly and *Renilla* luciferase provided by Promega

Instrument settings

	Omega series	CLARIOstar	PHERAstar *FS*
Detection mode	Luminescence		
Method	Well Mode Kinetic, Top optic		
Optic settings	Emission: lens	Emission: full range or Monochromator (520 – 620 nm)	Lumine-scence Optic module
Positioning delay	0.2 seconds		
Number of intervals	48		
Inverval time	0.5 seconds		
Injection start time	0 and 12 seconds		
Injection speed	220 or 230 µl/second		

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These experiments were performed as described in the Promega Instrumentation Certification documentation. 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 (3.0-12 seconds).
Range 2 - *Renilla* luminescence (14.5-23.5 seconds).

Results & Discussion

Criterion 1: Quenching of >10,000 Firefly/*Renilla*
Recombinant firefly luciferase exhibited quenching that was >10,000 fold (DLR requirements) (Figure 3). This was calculated by dividing blank corrected Firefly luminescence by blank corrected *Renilla* luminescence (no *Renilla* was used in this experiment).

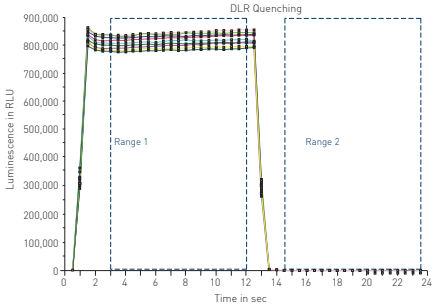

Fig. 3: **Criterion 1**- Graph showing Firefly luciferase quenching taken from MARS evaluation software (>10,000 fold [n=24]. Data measured on an Omega series reader using 3.05 ng/ml of recombinant Firefly luciferase.

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. The %CV values were below 5 % for all tested BMG LABTECH instrumentation.

Table 1: **Criterion 2** – Consistency has to be < 5 %.

%CV, n = 24	Consistency Part1		Consistency Part2	
	Firefly	*Renilla*	Firefly	*Renilla*
Omega series	2.3	2.5	2.2	2.0
CLARIOstar	2.0	1.5	0.5	2.3
PHERAstar *FS*	1.5	1.8	1.6	0.6

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 3 and therefore clearly within the criterion (Table 2).

Table 2: **Criterion 3** – Tubing Adsorption shows little change after 10 minutes, for n=12.

Omega series	Firefly		*Renilla*	
	Average	%CV	Average	%CV
RLU	1.055E7	2.5	3.711E6	1.8
RLU (after 10 min)	1.030E7	1.9	3.793E6	2.5
CLARIOstar	Firefly		*Renilla*	
	Average	%CV	Average	%CV
RLU	7.006E6	0.7	1.768E5	1.4
RLU (after 10 min)	6.91E6	0.5	1.762E5	2.0
PHERAstar *FS*	Firefly		*Renilla*	
	Average	%CV	Average	%CV
RLU	1.552E7	1.5	8.925E5	1.7
RLU (after 10 min)	1.536E7	1.1	1.536E7	1.8

Conclusion

The Omega series of microplate readers as well as the CLARIOstar and the PHERAstar *FS* from BMG LABTECH has been granted DLReady™ certification based on the results published in this application note.

DLReady™