Application Note

DUAL-LUCIFERASE® REPORTER SYSTEM WITH THE SIRIUS 2 TUBE LUMINOMETER

Abstract

Promega's Dual-Luciferase® Reporter (DLR™) Assay System is a widely used commercial reporter gene assay, employing firefly luciferase as the reporter for the promoter of interest and renilla luciferase as an internal control. In this application note, the Sirius 2 tube luminometer was validated for its compatibility with the DLR™ Assay System. The results demonstrate that the Sirius 2 passes all validation criteria and is DLReady™.

Introduction

Reporter genes are powerful tools for studying gene expression and are widely applied in biomedical, pharmaceutical, molecular biology, and biochemistry research.

The primary goal of a reporter gene assay is to analyses the promoter of a gene of interest, i.e., to investigate the regulation of its expression. This is typically achieved by linking the promoter to an easily measurable gene, such as luciferase, which catalyses a light-emitting reaction.

Luminescence-based reporter gene assays are especially popular due to several advantages:

- Very high sensitivity (10 to 1,000 times greater than absorbance- or fluorescence-based methods).
- Lack of endogenous luciferase activity in most cell types, minimizing background interference.
- Wide dynamic range.
- Fast and straightforward workflow.
- Relatively low costs.

To reduce experimental variability caused by random factors such as cell number, viability, or transfection efficiency, dual reporter systems are often employed. In these systems, two luciferases are expressed simultaneously: one under the control of the promoter of interest and the other driven by a constitutive promoter, serving as an internal control for normalization [1].

The Dual-Luciferase® Reporter (DLR™) Assay System from Promega is one of the most widely used dual reporter commercial solutions. It employs firefly luciferase as the reporter for the promoter of interest and renilla luciferase as the internal control. The assay consists of two steps: first, addition of the LAR II reagent to initiate the firefly luminescence measurement, followed by addition of the Stop & Glo® reagent, which quenches firefly activity and simultaneously activates the renilla luminescence [1].

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In this application note, the Sirius 2 tube luminometer is tested following the validation procedure from Promega for the DLR™ Assay System.

Sirius 2 tube luminometer

High performance luminescence measurement at your fingertips

Sirius 2 is a compact and highly sensitive tube luminometer. Light collection is maximized through both the close proximity of the sample to the detector and the use of optimized light reflectors surrounding the sample vial.

The luminometer is upgradeable with up to two injectors, making it perfect for flash assays, and can be equipped with an intuitive touch screen computer, enabling stand-alone operation.

The Sirius 2 supports all standard luminescence technologies including flash-type Luminescence, glow-type Luminescence, bioluminescence and chemiluminescence.

Materials

- Sirius 2 Touch-2 from Berthold Technologies (Id. Nr. 84018-03).
- Renilla luciferase, 0.78 mg/mL, from Promega (Part # E359).
- Firefly luciferase - QuantiLum® Recombinant Luciferase, 12.4 mg/mL, from Promega (Cat# E1701).
- Bovine Serum Albumin, Acetylated (BSA), 10 mg/mL, from Promega (Cat# R3961)
- Dual-Luciferase® Reporter Assay System from Promega (Cat# E1910)
- Luminescence tubes 5 mL, 12 x 75 mm, from Berthold Technologies (Id. Nr. 09778)
- Nuclease-Free Water from Promega (Cat# P1193)
- Pipettes and pipette tips (various volumes).

Methods

Reagents were prepared according to the manufacturer's instructions. To prepare the luciferase dilutions, a dilution buffer was prepared by mixing 1 mL of Passive Lysis buffer from the DLR™ kit with 0.5 mL of BSA 10 mg/mL and 3.5 mL of nuclease-free water.

In order for a luminometer to be validated for the DLR™ Assay System, the instrument has to pass 3 different tests:

1. Tubing adsorption: this test shows whether the tubing used in the instrument injectors has an effect on the DLR assay over time. 20 µL of 50:1 firefly:renilla solution are measured in 12 replicates. The test passes if signal after 10 minutes with reagents standing in the tubing is ≥95% of the signal before incubation.
2. Firefly luciferase quenching: this test shows if the injection system provides enough mixing for signal of firefly luciferase to be quenched. 20 µL of firefly luciferase 3.05 x 10^{-5} µg/µL are measured in 24 replicates. As there is no renilla luciferase, the signal of the 2nd measurement is unquenched firefly signal. The test passes if firefly signal after dispensing the Stop & Glo® reagent is quenched at least 10,000 times.
3. Consistency: this test shows if results are consistent in 24 replicates of 20 µL with 2 different firefly:renilla ratios (50:1 firefly:renilla and 50:1 renilla:firefly). The test passes if CV of the measurement is ≤5% both for firefly and renilla luciferases.

Instrument settings

The following settings were used in all tests (see Fig. 1):

1. Dispense 100 µL with injector 1 (LAR II).
2. Delay 2 s.
3. Luminescence measurement, time 10 s (firefly).
4. Dispense 100 µL with injector 2 (Stop & Glo®).
5. Delay 2 s.
6. Luminescence measurement, time 10 s (renilla).

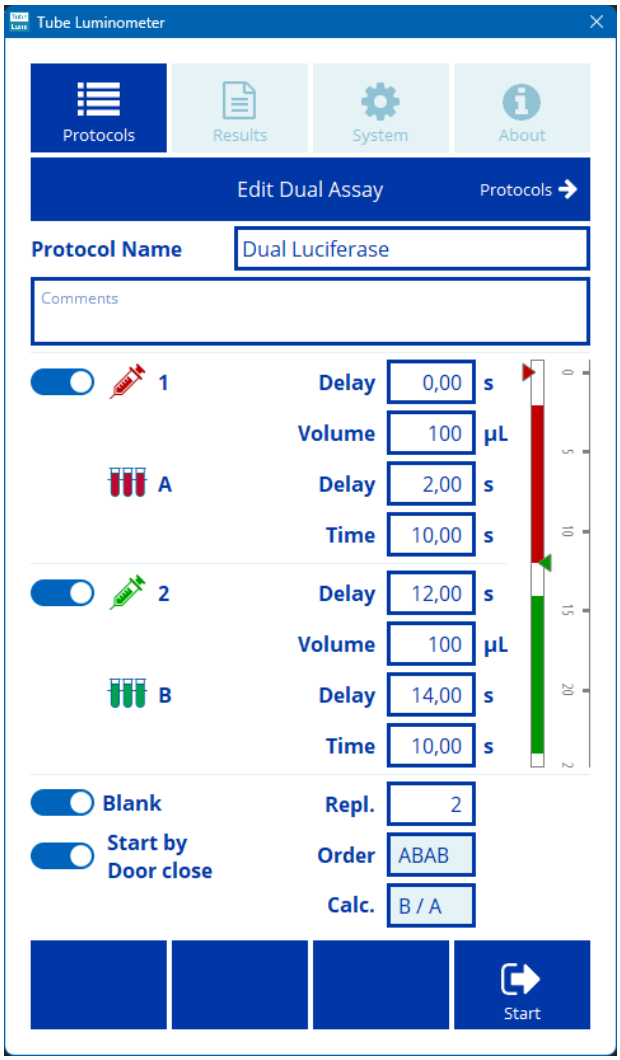

Figure 1: Instrument settings as programmed in the Sirius 2 software of the integrated touch screen computer. Delay of each event is counted from time=0, hence injector 2 starts at t=12 s and the 2nd measurement happens at t=14 s. Timing overview is represented at the right of the screen.

Results

Results of all 3 tests of the validation are summarized in **Table 1**.

Test 1 (adsorption test) passes if signal after 10 minutes with reagents standing in the tubing is ≥95% of the signal at time = 0. Results obtained with the Sirius 2 were 103.6% for the firefly measurements and 98.7% for the renilla measurements, indicating virtually no tubing adsorption.

Test 2 (quenching test) passes if quenching is at least 10,000. Quenching obtained with the Sirius 2 was above 70,000 (table 1), exceeding the required quenching by more than 7 times. Looking at the values of individual wells, the lowest quenching obtained was 30,497, also exceeding the required value of 10,000.

Test 3 (consistency test) passes if CV of the measurement is ≤5%. CV of the measurements performed with the Sirius 2 was <4.2% in all cases, with CV as low as 0.9% in some cases.

Test 1			**Test 3**		
	Firefly	Renilla	50:1 Firefly:Renilla	Firefly	Renilla
Average t=0	8,260,964	488,299	Average	9,053,731	467,034
Average t=10 min	8,556,572	482,146	Std. Dev	296,338	19,506
Activity (%)	**103.6**	**98.7**	CV (%)	**3.27**	**4.18**
Test 2			50:1 Renilla:Firefly	Firefly	Renilla
	Firefly	Renilla	Average	264,267	12,775,868
Average	6,278,993	84	Std. Dev	4,014	115,543
Quenching		**74,935**	CV (%)	**1.52**	**0.90**

Table 1: Results of tests 1, 2 and 3. Number of replicates: 12 in test 1, 24 in tests 2 and 3. Results in RLU/s unless otherwise indicated. Quenching is the average of Firefly/Renilla ratio of individual wells.

Conclusions

Taking all results into account, the Sirius 2 meets or exceeds all parameters required for the validation of the Dual-Luciferase Reporter® (DLR™) Assay System and is thus an excellent instrument to perform reporter gene assays using this system. This secures the DLReady™ certification for the Sirius 2; and consequently, it is listed by Promega at https://www.promega.com/en/resources/guides/lab-equipment-and-supplies/dlready-validated-luminometers/

References

1. Sherf, B.A., Navarro, S.L., Hannah, R.R., Wood, K.V. (1996). Dual-Luciferase™ Reporter Assay: An Advanced Co-Reporter Technology Integrating Firefly and Renilla Luciferase Assays. Promega Notes Magazine 57, 2-9.

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