

Application Note

DUAL-LUCIFERASE® REPORTER (DLR™) VALIDATION OF THE CENTRO LB 963

Abstract

The Dual-Luciferase Reporter® (DLR™) Assay System from Promega is a popular commercial assay using firefly luciferase as reporter for a promoter of interest and renilla luciferase as internal control reporter. Promega's DLR assay had been validated on the Centro LB 963 microplate luminometer.

Introduction

Reporter genes have become an invaluable tool in studies of gene expression. They are widely used in biomedical and pharmaceutical research and also in molecular biology and biochemistry.

The main purpose of the reporter gene assay is to investigate the promoter of a gene of interest, i.e., the regulation of its expression. This can be done by linking the promoter of interest to an easily detectable gene, such as the gene for firefly luciferase, which catalyses a reaction that produces light.

Reporter gene assays based on luminescence are very popular for several reasons:

- They have a high sensitivity (typically 10 to 10,000 times higher than methods based on absorbance or fluorescence).
- Most cell types do not have endogenous luciferase activity.
- Luminescence assays have a large dynamic range.
- They are quick to perform.
- Their costs are relatively low.

In order to minimize experimental variability caused by random factors (such as differences in cell number, cell viability or transfection efficiency), dual reporter systems can be used. In such systems, two different luciferase reporter enzymes are expressed simultaneously in each cell: one is controlled by the promoter of interest and the other one is controlled by a promoter that gives a stable expression and does not change with the experimental conditions, which is used as internal control for normalization [1].

The Dual-Luciferase Reporter® (DLR™) Assay System from Promega is a popular commercial assay using firefly luciferase as reporter for the promoter of interest and renilla luciferase as internal control reporter. In a first step, a reagent containing the substrate of firefly luciferase (LAR II reagent) is dispensed, and the firefly luminescence is measured; in a second step a reagent is dispensed, which quenches the firefly luminescence and starts the renilla luminescence (Stop & Glo® reagent), and the renilla luminescence is measured [1].

Authors

Francesc Felipe, Timo Staab
Berthold Technologies GmbH
www.berthold.com/bio

Centro LB 963 Microplate Luminometer

The Centro is a high-performance, easy to use microplate luminometer for both, flash, and glow luminescence applications.

The optimized design provides excellent performance and flexibility:

- Superior sensitivity (<math><1.8 \text{ zmol}</math> firefly luciferase)
- Negligible crosstalk (- Built-in shaker
- JET-injectors
- Temperature control (model-dependent)
- Ergonomic design
- Automation compatibility

The Centro is ideally suited for all luminescent reporter gene assays, immunoassays (LIA, ILMA), cell-based, and biochemical assays.



To meet your compliance requirements, a set of validation tools and optional software providing 21 CFR part 11 compliance are available.

Materials

- Centro LB 963 Microplate Luminometer from Berthold Technologies (Id. Nr. 70325-10).
- 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)
- 96 well white plates from Costar (Cat # 3912).
- Nuclease-Free Water from Promega (Cat# P1193)
- Pipettes and pipette tips (various volumes).

Instrument settings

The following settings were programmed in the ICE software and used in all tests:

1. Dispense 100 μL LAR II, injector 1, speed: middle, by well.
2. Delay 2 s, by well.
3. Endpoint luminescence, counting time 10 s, by well.
4. Dispense 100 μL Stop & Glo®, injector 2, speed: middle, by well.
5. Delay 2 s, by well.
6. Endpoint luminescence, counting time 10 s, by well.

Methods

Reagents and luciferase dilutions were prepared according to the manufacturer's instructions.

In order for a microplate reader 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. The test passes if signal after 10 minutes with reagents standing in the tubing is $\geq 95\%$ 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. 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 with 2 different firefly:renilla ratios (50:1 firefly:renilla and 50:1 renilla:firefly). The test passes if CV of the measurement is $\leq 5\%$ both for firefly and renilla luciferases.

Results and conclusion

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

Test 1 passes if signal after 10 minutes with reagents standing in the tubing is $\geq 95\%$ of the signal at time = 0. Results obtained with the Centro were 98.5% for the firefly measurements and 102.7% for the renilla measurements, indicating virtually no tubing adsorption.

Test 2 passes if quenching is at least 10,000. Quenching obtained with the Centro was above 100,000 (table 1), exceeding the required quenching by more than 10 times. Looking at the values of individual wells, the lowest quenching obtained was 23,435 (data not shown), also exceeding the required value of 10,000.

Test 3 passes if CV of the measurement is $\leq 5\%$. CV of the measurements performed with the Centro was $< 3\%$ in all cases.

Taking all results into account, the Centro 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.

Test 1		
	Firefly	Renilla
Average t=0	123,695,545	3,536,743
Average t=10 min	121,830,671	3,631,599
Activity (%)	98.5	102.7
Test 2		
	Firefly	Renilla
Average Quenching	132,401,691	1,679
		109,811
Test 3		
50:1 Firefly:Renilla	Firefly	Renilla
Average	131,495,776	3,526,940
Std. Dev	3,118,096	101,652
CV (%)	2.37	2.88
50:1 Renilla:Firefly	Firefly	Renilla
Average	2,591,584	64,433,715
Std. Dev	56,652	3,211,533
CV (%)	2.19	1.95

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 unless otherwise indicated. Quenching is the average of Firefly/Renilla ratio of individual wells.

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.

Not for use in diagnostic procedures.

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Berthold Technologies GmbH & Co. KG

Calmbacher Straße 22

75323 Bad Wildbad

GERMANY

Phone: +49 7081 177 0

Email: bio@berthold.com



www.berthold.com