Application Note

DUAL-LUCIFERASE® REPORTER (DLR™) VALIDATION OF THE TRISTAR 3

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 Tristar 3 multimode microplate reader.

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:

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- 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].

Tristar 3 Multimode Microplate Reader

The Tristar 3 is a user-friendly and affordable filter-based multimode plate reader. Its ONE-4-ALL optics Our patented ONE-4-ALL optics have been optimised to combine the stability and user-friendliness of a multimodal optical system with the sensitivity and versatility of dedicated optical devices.

The Tristar 3 Multimode Microplate Reader provides you with application flexibility for today, tomorrow, and beyond to master your changing plate reading applications:

- High sensitivity luminescence
- BRET
- Absorbance (UV/VIS)
- Fluorescence
- FRET

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

Materials

- Tristar 3 Multimode Microplate Reader from Berthold Technologies (Id. Nr. 69173-10).
- 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: 4, 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: 4, 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 ≥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 ≤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 ≥95% of the signal at time = 0. Results obtained with the Tristar 3 were 104.3% for the firefly measurements and 100.8% for the renilla measurements, indicating virtually no tubing adsorption.

Test 2 passes if quenching is at least 10,000. Quenching obtained with the Tristar 3 was above 900,000 (table 1), exceeding the required quenching by more than 90 times. Looking at the values of individual wells, the lowest quenching obtained was 27,951 (data not shown), also exceeding the required value of 10,000.

Test 3 passes if CV of the measurement is ≤5%. CV of the measurements performed with the Tristar 3 was <5% in all cases.

Taking all results into account, the Tristar 3 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	130,812,339	3,536,743
Average t=10 min	136,416,868	3,631,599
Activity (%)	**104.3**	**100.8**
Test 2		
	Firefly	Renilla
Average	144,053,616	552
Quenching		**984,225**
Test 3		
50:1 Firefly:Renilla	Firefly	Renilla
Average	50,995,696	2,941,702
Std. Dev	1,519,364	133,033
CV (%)	**2.97**	**4.52**
50:1 Renilla:Firefly	Firefly	Renilla
Average	1,080,711	108,166,726
Std. Dev	26,599	2,578,779
CV (%)	**2.46**	**2.38**

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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