Application Note

DUAL-LUCIFERASE® REPORTER (DLR™) VALIDATION OF THE TRISTAR 5

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 5 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 5 Multimode Microplate Reader

The Tristar 5 is a modular high-performance microplate reader equipped with FlexTec Optics, offering you the best of two worlds: independent, user-selectable filters and monochromators on both, the excitation and emission side, for any measurement. This guarantees both, flexibility and sensitivity whenever you need it.

The Tristar 5 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
- TRF, TR-FRET and HTRF®
- FP
- AlphaScreen®

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

Materials

- Tristar 5 Multimode Microplate Reader from Berthold Technologies (Id. Nr. 69185-15).
- 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 5 were 99.6% for the firefly measurements and 98.0% for the renilla measurements, indicating virtually no tubing adsorption.

Test 2 passes if quenching is at least **10,000**. Quenching obtained with the Tristar 5 was above 390,000 (table 1), exceeding the required quenching by more than 39 times. Looking at the values of individual wells, the lowest quenching obtained was 76,948 (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 5 was <5% in all cases.

Taking all results into account, the Tristar 5 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	11,921,041	121,261
Average t=10 min	11,874,673	118,843
Activity (%)	**99.6**	**98.0**
Test 2		
	Firefly	Renilla
Average	16,183,178	57
Quenching		**392,910**
Test 3		
50:1 Firefly:Renilla	Firefly	Renilla
Average	11,427,319	113,327
Std. Dev	241,678	4,659
CV (%)	**2.11**	**4.11**
50:1 Renilla:Firefly	Firefly	Renilla
Average	221,718	5,075,557
Std. Dev	4,683	112,811
CV (%)	**2.11**	**2.22**

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.

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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