Application Note

DUAL-LUCIFERASE® REPORTER SYSTEM WITH THE JUNIOR TUBE LUMINOMETER

HIGH PERFORMANCE IN A SMALL FORMAT

Abstract

The Dual-Luciferase® Reporter (DLR™) Assay System from Promega is a popular commercial reporter gene assay using firefly luciferase as reporter for the promoter of interest and renilla luciferase as internal control reporter. In this application note, the Junior portable tube luminometer is used to measure samples with a broad range of firefly:renilla ratios. Results show that the Junior offers high sensitivity and no interference from high firefly concentrations.

Introduction

Reporter genes have become a very valuable 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 a luciferase, which catalyses a reaction that produces light.

Reporter gene assays based on luminescence are very popular for a variety of reasons:

- They have high sensitivity (typically 10 to 1,000 times higher than methods based on absorbance or fluorescence).
- Most cell types do not have endogenous luciferase activity that could interfere with the assay.
- Luminescence-based 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: 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

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(Stop & Glo® reagent), and the renilla luminescence is measured [1].

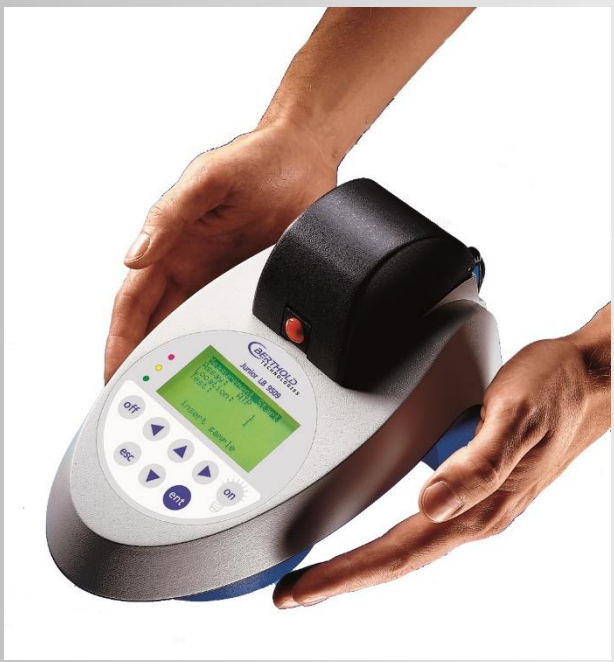
One critical point for the performance of the assay is the quenching of the signal of the firefly luciferase: if there is residual luminescence from the firefly luciferase when the reading of the renilla luciferase is taken, the signal of the internal control will be overestimated, and this could lead to wrong results. In this application note, the Junior portable tube luminometer is used to measure samples with a broad range of firefly:renilla ratios using the DLR™ assays system to test its sensitivity, linearity and quenching efficiency.

Junior portable tube luminometer

High performance luminescence measurement, wherever you need it

The Junior is a small portable tube luminometer which can be used for all common applications using glow luminescence. Excellent performance, small size, low weight and battery-powered mode make it an ideal partner whenever mobility counts - in the laboratory, on site, or outdoors.

The Junior can be used for a wide range of fields, including biomedical research, hygiene monitoring, process control in biotechnology, environmental monitoring (e.g. water quality) and others.



Materials

- Junior Portable Tube Luminometer from Berthold Technologies (Id. Nr. 32526-10).
- 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)
- Lumivials 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.

A dilution series of firefly luciferase with concentrations ranging from 10^{-19} up to 10^{-15} mol/µL was prepared and was mixed 1:1 with a solution of renilla luciferase 10^{-19} mol/µL; this gives firefly:renilla ratios from 10000:1 to 1:1. 20 µL of this mix were transferred to lumivials in triplicate. The final quantity of renilla luciferase per tube was 10^{-18} mol in all tubes, and the quantity of firefly luciferase was 10^{-18}, 10^{-17}, 10^{-16}, 10^{-15} and 10^{-14} mol/tube. Blank tubes containing dilution buffer only were also measured in triplicate.

To perform the measurement, the following procedure was repeated for each tube: first, 100 µL of LAR II were pipetted into the tube, the tube was mixed using a vortex for 2 seconds and luminescence was measured in the Junior for 10 seconds (firefly luciferase reading); then, 100 µL of Stop & Glo® reagent were pipetted in the tube, the tube was mixed using a vortex for 2 seconds and luminescence was measured in the Junior for 10 s (renilla luciferase reading). For each tube, the firefly signal was divided by the renilla signal to calculate the firefly/renilla ratio.

Results

The firefly/renilla ratios show excellent linearity in the whole concentration range tested, with a correlation coefficient R^2 of 0.9999 (Fig. 1).

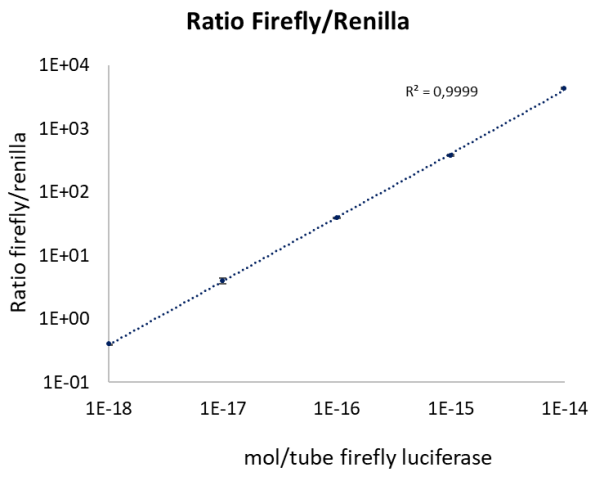


Figure 1. Firefly/renilla ratios of the different mixes. Error bars represent standard error of the mean. All data points measured in triplicate.

Looking at the readings of renilla luciferase, results show complete quenching of the firefly luciferase signal (Fig. 2): in the tube with the highest firefly:renilla ratio (10000:1), firefly signal reaches 518140 RLU (data not shown), but even this strong signal is totally quenched, as its renilla signal is not significantly different from the tube with the lowest firefly:renilla ratio (1:1), in which the firefly signal is 63 RLU only.

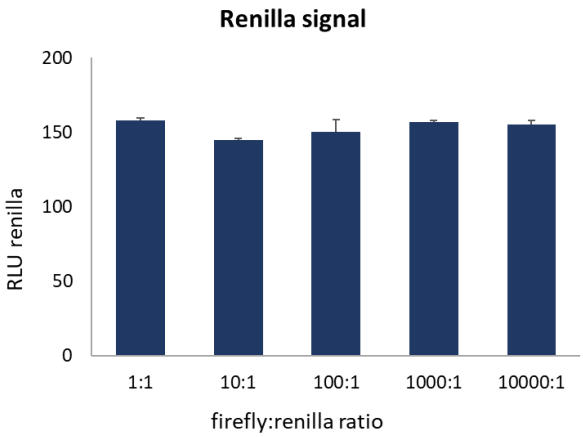


Figure 2. Renilla signal (in Relative Light Units) at different firefly:renilla ratios. Error bars represent standard error of the mean. All data points measured in triplicate.

Discussion and conclusions

The Dual-Luciferase® Reporter Assay is a very popular tool for the study of the regulation of gene expression and, in the conditions tested, the measurement of the second signal (renilla luciferase) didn't show any interference from the first signal (firefly luciferase), even at firefly:renilla ratios so high as 10000:1.

The Junior tube luminometer is the smallest and most affordable tube luminometer from Berthold's instrument range. While it lacks reagent injectors, that would reduce labour and improve the reproducibility of the assay, our results show that it is very suitable to measure the (DLR™) Assay System, as demonstrated by its excellent linearity and good sensitivity in the range of concentrations and firefly:renilla ratios tested. This makes it an affordable but robust solution to investigate the regulation of gene expression using the Dual-Luciferase® Reporter Assay System from Promega.

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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