Dual-Glo™ Luciferase Assay System: A Homogeneous Dual-Reporter System

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Abstract

Promega has developed a new, homogeneous reporter assay, the Dual-Glo™ Luciferase Assay System, for monitoring both firefly and Renilla *luciferases expressed in mammalian cells in 96- and 384-well plates. The new assay system integrates cell lysis and luminescence chemistry, and the luminescence kinetics have been extended over several hours. The homogeneous assay format allows dual-reporter assays to be more easily and rapidly performed by reducing sample processing requirements and eliminating the need for reagent injectors in luminometers.*

Introduction

Although reporter genes are widely used for rapid evaluation of cellular physiology (1,2), a single reporter may not convey sufficient information for reliable interpretation of the experimental data. For this reason, dual reporters are commonly used, most notably the bioluminescent firefly and *Renilla* luciferase genes. Promega recently introduced the Dual-Glo™ Luciferase Assay System[a,b,c] (3) for dual reporter measurements in a homogeneous assay format. Like Promega's Dual-Luciferase® Reporter Assay System[a,b,c], the new Dual-Glo™ Assay allows sequential measurement of both firefly and *Renilla* luciferases from one sample. However, the kinetics of the luminescent reactions have been extended to allow for processing multiple samples before initiating measurements. Reagent can be added to all samples in a multiwell plate, or even to a stack of multiwell plates, before placing the plates in a luminometer. To further simplify sample processing, the lytic components of the assay have been combined with the luminescent chemistry to yield an integrated assay formulation. Consequently, the Dual-Glo™ Assay can be performed by adding the reagents directly to cells in culture medium and measuring the resulting luminescence.

Reporter genes offer an excellent means for studying complex genetic regulatory networks. However, this complexity can also make it difficult to isolate and characterize a specific physiological pathway without interference from other elements within the system. The significance of this interference on reporter responses can be realized only through a properly configured reference, typically from a secondary reporter. Dual reporters facilitate the extraction of useful data by differentiating genetic responses of interest from non-relevant influences. Such influences may include "edge effect" in multiwell plates, transfection efficiency in transiently transfected cells, and other sources of interference, often recognized simply as "noise" in the system.

Interference in the reporter response can also arise when underlying genetic events are masked by physiological factors such as cell viability. This is problematic for distinguishing genetic downregulation from cytotoxicity, particularly when using specific downregulation as a means of identifying novel receptor antagonists, because a reduction of reporter expression can be confused with cytotoxicity.

Distinguishing Downregulation from Cytotoxicity

To model this circumstance, single- and dual-reporter measurements were made in cells expressing *Renilla* luciferase under control of the Tet-Off promoter and firefly luciferase under control of the CMV promoter (Figure 1). Reporter activity was measured after adding titrated amounts of doxycycline or G418 antibiotic to the cells. Doxycycline is expected to specifically downregulate the *Renilla* expression coupled to the Tet-Off promoter, while G418 is expected to kill the cells. Because both compounds reduce *Renilla* luminescence with increasing dose, it is not possible to distinguish specific genetic regulation from cell death using a single reporter. This is demonstrated in Panel A, where only *Renilla* luciferase activity was assayed. In contrast, by using the firefly luciferase as an internal reference, the distinction between genetic response and cell death is clear. Doxycycline reduces only *Renilla* luminescence, but cell death caused by G418 reduces the luminescence of both reporters. This distinction can be readily displayed as the ratio of *Renilla* to firefly luminescence. In Panel B, the *Renilla* luciferase activity is normalized to the co-transfected firefly luciferase control.

The Dual-Glo™ Assay can be performed by adding the reagents directly to cells in culture medium.

Summary

Experimental strategies involving dual reporters have become increasingly common, preferred for their ability to provide reliable and meaningful data. Reporter analyses also are increasingly being performed in multiwell plates. The culmination of these trends is high-throughput screening, where huge numbers of samples are quantitatively analyzed. The Dual-Glo™ Assay System was developed to support these requirements by providing a simple, homogeneous means of quantifying both the firefly and *Renilla* luciferases from mammalian cells in culture medium.

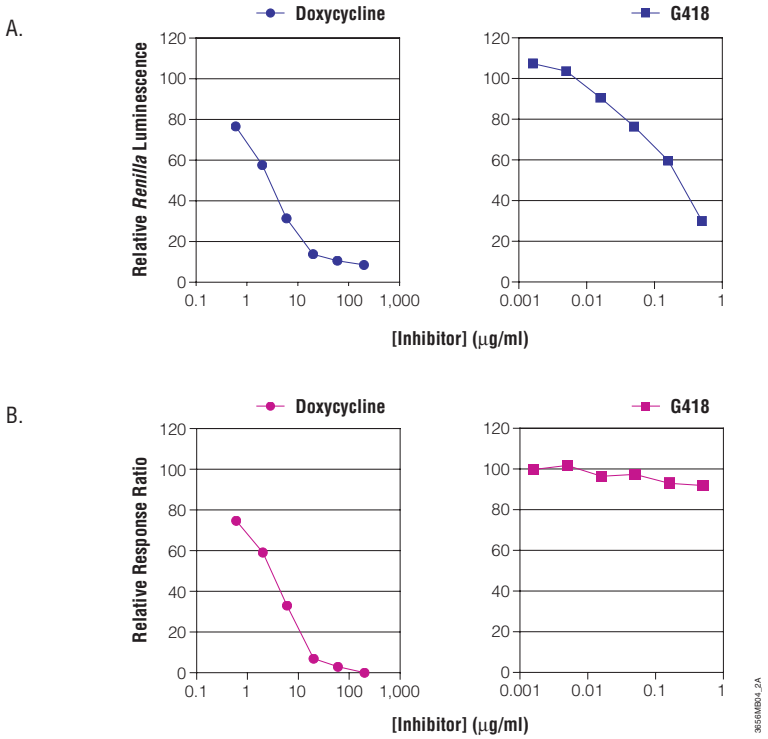

Figure 1. Differentiating genetic downregulation from cytotoxicity. CHO cells were transiently transfected with *Renilla* luciferase under the control of the Tet-Off promoter and firefly luciferase under the control of the CMV promoter. Cells were titrated with a specific inhibitor of *Renilla* luciferase expression (doxycycline) or a cytotoxic agent (G418 antibiotic). **Panel A** illustrates that the output from a single reporter is similar under both inhibitors, because both test compounds yield diminished reporter luminescence. The y-axis shows relative *Renilla* luminescence as a percent of sample without inhibitor. In contrast, **Panel B** shows that a dual-reporter system can clearly distinguish between specific downregulation of the gene and cytotoxicity. The output is recorded as a relative response ratio (RRR), where the sample, negative and positive controls are reported as the ratio of *Renilla* luminescence to firefly luminescence. Measurements of the relative response ratios were also more precise than the measurements of a single reporter (average relative standard deviation of 6.5% in Panel B compared with 13% in Panel A). RRR= [sample – negative control] / [positive control – negative control], as a percent.

References

1. Alam, J. and Cook, J.L. (1990) Reporter genes: Application to the study of mammalian gene transcription. *Anal. Biochem.* **188**, 245–54.
2. Wood, K.V. (1991) In: *Bioluminescence and Chemiluminescence: Current Status*, Stanley, P. and Kricka, L., eds. John Wiley and Sons, Chicester, NY 543.
3. Hawkins, E. *et al.* (2002) Dual-Glo™ Luciferase Assay System: Convenient dual reporter measurements in 96- and 384-well plates *Promega Notes* **81**, 22–26.

Protocols

Dual-Glo™ Luciferase Assay System Technical Manual #TM058, Promega Corporation

www.promega.com/tbs/tm058/tm058.html

Ordering Information

Product	Size	Cat.#
Dual-Glo™ Luciferase Assay System	10ml	E2920
	100ml	E2940
	10 × 100ml	E2980

(a)Certain applications of this product may require licenses from others.
(b)U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289, 5,814,471, Australian Pat. No. 649289 and European Pat. No. 0 553 234 have been issued to Promega Corporation for a firefly luciferase assay method, which affords greater light output with improved kinetics as compared to the conventional assay. Other patents are pending.
(c)U.S. Pat. No. 5,744,320 and Australian Pat. No. 721172 have been issued to Promega Corporation for quenching reagents and assays for enzyme-mediated luminescence. Other patents are pending.