Get Glowing Results from Your Assays

A Complete Solution for Measuring Biological and Cellular Activities: GloMax™ Luminometers and Bioluminescent Assays

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Abstract

Beginning with firefly luciferase reporter gene assays, Promega has a long history of producing assays in which bioluminescence is the output signal. We have expanded our luminescent technology to include a wide variety of assays for measuring different biochemical or cellular activities including apoptosis, cell viability, kinase activity and toxicology. Here we introduce the GloMax™ Integrated Luminescence Systems. The GloMax™ Systems are composed of reagents, instruments, software and protocols all with a single, comprehensive support system. Because of the excellent sensitivity and broad dynamic range of the GloMax™ 96 Luminometers, these are state-of-the-art, yet easy-to-use instruments for measuring any luminescent signal. They are ideally suited to measure bioluminescent assays available from Promega. Here we describe the use of the GloMax™ Luminometer to measure cell viability and caspase activity to highlight the integration between high-performance instrumentation and bioluminescent assays.

These luminometers are built for one thing—capturing the highest quality data possible with the most user-friendly interface.

Bioluminescent Assays

Bioluminescence has long been recognized as an optimal method for measuring biological activities (1–3). The hallmark bioluminescent assay is the luciferase gene reporter assay for quantitative measurement of gene expression. The original firefly luciferase assay system yields linear results over several orders of magnitude, and low background luminescence is found in the host cells or assay chemistry. Promega has developed a suite of bioluminescent assays that maintain this high quality but measure other biochemical or cellular activities of interest to the biological researcher (Table 1). Furthermore, these assays are easy-to-use and adaptable to different throughput needs.

Figure 1. The GloMax™ Luminometry Systems. Bioluminescent assay systems and reagents are available separately (Table 1).

Table 1. Bioluminescent Assays and Reagents Available from Promega for Use with the GloMax™ Luminometers.

Assay Type	Products Available for Use with GloMax™ Systems
Reporter Assays	Luciferase Assay Systems Bright-Glo™ Luciferase Assay System Steady-Glo® Luciferase Assay System *Renilla* Luciferase Assay System EnduRen™ Live Cell Substrate (*Renilla*) ViviRen™ Live Cell Substrate (*Renilla*) Beta-Glo® Assay System
Dual-Reporter Gene Assays	Dual-Luciferase® Reporter Assay System Dual-Glo™ Luciferase Assay System
Cell Viability Assay	CellTiter-Glo® Cell Viability Assay BacTiter-Glo™ Microbial Cell Viability Assay
Apoptosis Assays	Caspase-Glo® 3/7 Assay Caspase-Glo® 8 Assay Caspase-Glo® 9 Assay
Other Protease Assays	Calpain-Glo™ Assay DPPIV-Glo™ Assay
Kinase Assays	Kinase-Glo® Luminescent Kinase Assay Kinase-Glo® Plus Luminescent Kinase Assay
ADME Assays	P450-Glo™ CYP450 Assay Systems Pgp-Glo™ Assay Systems MAO-Glo™ Assay System

The GloMax™ 96 Microplate Luminometer

To fully gain the benefits of bioluminescence, a luminometer is required to measure sample activity. A typical luminometer consists of a light-sensitive photomultiplier tube (PMT) in a light-tight exterior. The PMT is situated close to the light-emitting sample for maximum sensitivity and collects signal over the entire spectrum of light. The advent of the multifunction reader that can read absorbance, fluorescence and luminescence has resulted in flexible instrumentation, but this instrumentation is not usually optimally designed for the demands of reading luminescence. Often sensitivity and dynamic range are compromised by designs that use long path lengths for filter wheels and other assemblies required for other types of measurement. The result is that extremely bright or dim samples will not be read appropriately by these instruments, and the data will be compromised. The GloMax™ Luminometers take full advantage of bioluminescence measurement. These luminometers are built for one thing: capturing the highest quality data possible with the most user-friendly interface.

The majority of the Promega bioluminescence assays feature an "add-mix-read" homogeneous format. The GloMax™ Luminometers come preloaded with protocols for reading the Promega assays, so no programming or optimization of the instrument is required. However, the flexible interface allows you to create your own methods to keep the instrument adaptable and able to meet your experimental needs.

In addition, the GloMax™ Luminometers are simple to maintain. Tubing paths for the injector system are visible, so that issues that might be common on some instruments, such as bubbles in the lines, can be detected before they cause problems. If bubbles or injector contamination should occur, the tips and tubing are all readily replaceable by the user.

Finally, Promega provides the same great level of technical support for the GloMax™ Integrated Luminescence System that we do for our reagents. Our global network of Technical Services Scientists is available to assist you whether you have questions about experimental design, the use of our reagents, or the GloMax™ Luminometers.

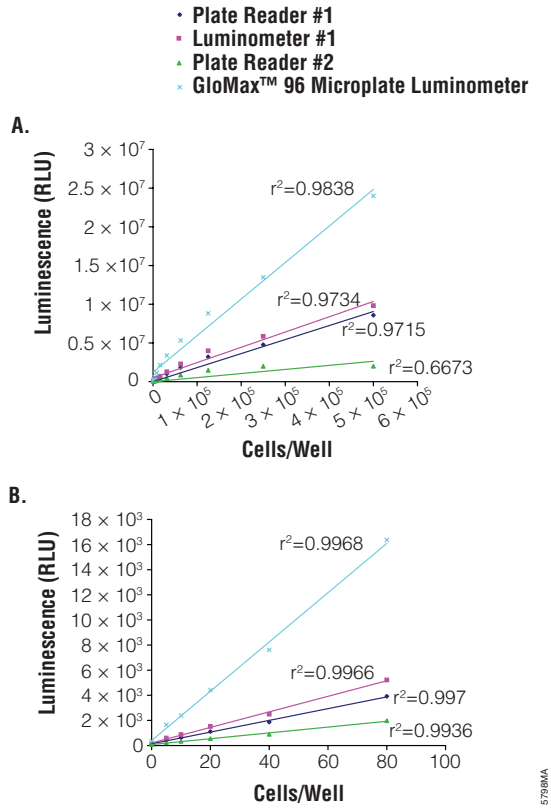

Figure 2. Demonstration of superior signal range obtained with GloMax™ 96 Microplate Luminometers. K562 cells were titrated from 0–500,000 cells/well, and viability was assessed using the CellTiter-Glo® Luminescent Cell Viability Assay according to the protocol provided in Technical Bulletin #TB288. Assay results were measured with either a GloMax™ 96 Microplate Luminometer or one of two different high-end multifunction plate readers. In addition readings were also made using a second, dedicated luminometer. **Panel A.** Best fit lines at the highest range of cell number. **Panel B.** Best fit lines for the lowest range of cell number.

Luminescent Cell Viability and Caspase Assays with the GloMax™ 96 Microplate Luminometer

To demonstrate the results achieved when a high-performance Promega bioluminescent assay is used on an instrument capable of properly measuring the entire signal range from the assay, the CellTiter-Glo® Luminescent Cell Viability Assay was run on a titration of K562 cells (0–500,000/well) and measured either with the GloMax™ Microplate Luminometer or one of two high-end multifunction plate readers. In addition, bioluminescence measurements were also made using a second dedicated luminometer.

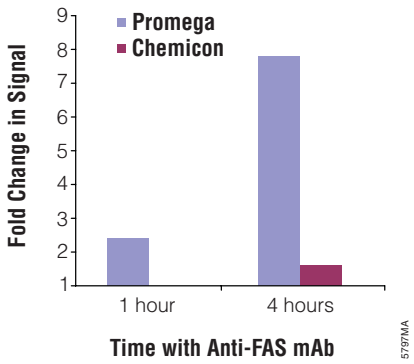

Figure 3. Fold change in caspase-3/7 signal when comparing untreated Jurkat cells to those treated for either 1 or 4 hours with anti-Fas monoclonal antibody. The results are the average of replicates of 6 for each treatment and time point.

The GloMax™ 96 Microplate Luminometer is able to measure as few as five viable cells in a white 96-well plate (Figure 2). Furthermore, at a density of 500,000 cells/well, the signal is in the linear range using the GloMax™ instrument (Figure 2). In comparison, the multifunction readers do not have the same capacity to resolve signal above background at low cell numbers, and they cannot cover the same dynamic range of viable cells (Figure 2). These results reflect the design of the multifunction plate readers, which are not optimized to read luminescent signals because of longer path lengths for filter wheels and other assemblies required for different types of measurements. Thus the GloMax™ Luminometer, designed specifically to read luminescence, provides the greatest signal range combined with sensitive detection.

To compare the performance of the GloMax™ 96 Microplate Luminometer to the multifunctional plate readers and to highlight the sensitivity of the Promega luminescent caspase assays, an apoptosis experiment was performed. We used a common model system for studying cell death in which apoptosis was induced in Jurkat cells by incubation with 400ng/ml anti-Fas mAb (clone CH-11) or vehicle for one or four hours (4). Following incubation with or without the anti-Fas mAb, caspase 3/7 activity was measured using a luminescent assay (Caspase-Glo® 3/7 Assay from Promega or CleavaLite® Caspase-3 Activity Assay Kit from Chemicon). The CleavaLite® Assay uses a *Renilla* luciferase mutant containing the caspase cleavage site, DEVD. Upon cleavage it exhibits decreased luminescence. The Caspase-Glo® Assay uses the luminogenic caspase-3/7 substrate, Z-DEVD-aminoluciferin. Upon cleavage of the substrate by caspase, aminoluciferin is liberated and consumed by luciferase, generating luminescence. The assays were performed following the manufacturers' directions in 96-well white plates in replicates of six and read using the the GloMax™ 96 Microplate Luminometer.

The results are shown in Figure 3 as fold change of caspase activity in anti-Fas mAb-treated cells over vehicle-treated cells at the one- and four-hour time points for each assay. The combination of the Caspase-Glo® 3/7 Assay and the GloMax™ 96 Microplate Luminometer gave the most sensitive results. Apoptosis induction in the Jurkat cells could be easily detected as early as one hour after treatment with the anti-Fas mAb. The CleavaLite® Caspase-3 Assay did not detect significant induction of caspase activity even at the four-hour time point. Thus the Caspase-Glo® chemistry, coupled with the GloMax™ 96 Microplate Luminometer, allowed sensitive and early detection of caspase-3/7 activity in this model system. The Caspase-Glo® Assay and GloMax™ Luminometer were also a more sensitive combination than the fluorescent Homogeneous Caspase Assay from Roche Applied Science read on a multifunctional plate reader (data not shown).

Summary

The combination of Promega bioluminescent assays and the wide dynamic range of the GloMax™ 96 Microplate Luminometer allows sensitive and linear detection of luminescent signals far superior to that achieved using other assays and detection instruments, particularly multifunctional readers. The Promega bioluminescent assays provide sensitive signal and low background in easy-to-use formats. The GloMax™ Luminometers in combination with high-performance bioluminescent assays from Promega provide researchers with the ability to generate high-quality data.

References

1. Wood, K.V. (1995) *Curr. Biol.* **6**, 50–8.
2. Naylor, L.H. (1999) *Biochem. Pharmacol.* **58**, 749–57.
3. Wood, K.V. (2004) *Progress in Biomedical Optics and Imaging.* Nicolau, D.V. and Raghavachari, R. eds. SPIC–The International Society for Optical Engineering, Bellingham, 66–77.
4. Niles, A., Moravec, R. and Riss, T. (2004) *Cell Notes* **9**, 11–14.

Protocols

- *GloMax™ 96 Microplate Luminometer Technical Manual* #TM278, Promega Corporation.
  www.promega.com/tbs/tm278/tm278.html
- *GloMax™ 20/20 Luminometer Technical Manual* #TM276, Promega Corporation.
  www.promega.com/tbs/tm276/tm276.html
- *CellTiter-Glo® Luminescent Cell Viability Assay Technical Bulletin* #TB288, Promega Corporation.
  www.promega.com/tbs/tb288/tb288.html
- *Caspase-Glo® 3/7 Assay Technical Bulletin #*TB323, Promega Corporation.
  www.promega.com/tbs/tb323/tb323.html

Ordering Information

Product	Size	Cat.#
GloMax™ 96 Microplate Luminometer		E6501
GloMax™ 96 Microplate Luminometer with Single Reagent Injector		E6511
GloMax™ 96 Microplate Luminometer with Dual Reagent Injectors		E6521
GloMax™ 20/20 Luminometer		E5311
GloMax™ 20/20 Luminometer with Single Auto-Injector		E5321
GloMax™ 20/20 Luminometer with Dual Auto-Injector		E5331

Related Products

Product	Size	Cat.#
CellTiter-Glo® Luminescent Cell Viability Assay	10ml	G7570
	10 × 10ml	G7571
	100ml	G7572
	10 × 100ml	G7573
Caspase-Glo® 3/7 Assay*	2.5ml	G8090
	10ml	G8091
	10 × 10ml	G8093
	100ml	G8092

*For Laboratory Use.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

Related Citations

Ren, Y.G. *et al.* (2004) Differential regulation of the TRAIL death receptors DR4 and DR5 by the signal recognition particle. *Mol. Biol. Cell* 15, 5064–74.

Differences in the activation of apoptotic pathways by the TRAIL death receptors DR4 and DR5 were investigated. An siRNA screen was performed on HCT15 cells in 384-well plates to find genes that influence DR4- or DR5-mediated apoptosis. After induction of apoptosis using anti-DR4 or DR5 cross-linked antibodies, cell viability was assessed using the CellTiter-Glo® Reagent. The study showed that the Signal Recognition Particle (SRP) plays a major role in DR4- but not DR5-mediated apoptosis. To investigate further, stable HeLa cell lines were generated that expressed short hairpin RNA (shRNA) directed against SRP. The effects of various inducers of apoptosis were tested in these cell lines. Caspase activation was measured using the Caspase-Glo® 3/7 reagent.

Petty, W.J. *et al.* (2004) Epidermal growth factor receptor tyrosine kinase inhibition represses cyclin D1 in aerodigestive tract cancers. *Clin. Cancer Res.* 10, 7547–54.

The CellTiter-Glo® Luminescent Cell Viability Assay was used to examine the proliferation of BEAS-2B (immortalized human bronchial epithelial [HBE]) cells after treatment with various concentrations of erlotinib, an inhibitor of the mitogenic effects of EGF. For these experiments, 3,000 cells/well were seeded in 96-well plates and incubated for 72 hours with various concentrations of erlotinib. Other lung cell carcinoma cell lines (A549, H226, H358, and H441) were also tested for proliferation in the presence of erlotinib using the CellTiter-Glo® Assay System. The researchers used a luciferase construct that contained the cyclin D1 promoter to test the effects of erlotinib on cyclin d1 regulation. This construct, along with the pRL-TK vector, were co-transfected into BEAS-2B cells. Groups of cells were then treated with or without EGF and erlotinib and assayed for luciferase activity using the Dual-Luciferase® Reporter Assay System.

Additional peer-reviewed articles citing the use of Promega bioluminescent assays can be found at: **www.promega.com/citations/**