Bright-Glo™ and Steady-Glo™ Luciferase Assay Systems: Reagents for Academic and Industrial Applications

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The Bright-Glo™[(a)] and Steady-Glo™[(a,b)] Luciferase Assay Systems have been designed to meet the needs of virtually any research application of the firefly luciferase reporter gene. These reagents have been optimized for homogeneous or nonhomogeneous assay formats, manual or automated sample processing, small-scale analysis to ultrahigh-throughput screening. Assays may be performed in a single step by adding the reagent into the culture medium containing cells expressing luciferase. The reagents provide exceptional assay precision with the highest sensitivity available. Characteristics of each reagent are further described to assist researchers in choosing the best reagent for their experiments.

Introduction

Research applications using firefly luciferase have diversified tremendously over the past decade. Experimental strategies today may involve the analysis of a few samples per day, or as many as several thousand samples per hour. Individual samples may be measured using inexpensive luminometers, or thousands of samples may be measured simultaneously using high-end CCD luminometers. To support this wide range of applications, we have developed the Bright-Glo™ and Steady-Glo™ Luciferase Assay Systems. These reagents are designed for both homogeneous and nonhomogeneous assay formats, and provide the highest sensitivity available. They are intended to fulfill the needs of both academic and industrial scientists.

The Bright-Glo™ and Steady-Glo™ Reagents provide enhanced assay performance and flexibility compared to Promega's Luciferase Assay System[(a)], introduced in 1990. Although the Luciferase Assay System has long been the standard in routine laboratory analyses, its performance was not optimized for measurements in microplates. Bright-Glo™ and Steady-Glo™ Reagents are designed to enable efficient and precise quantitation in 96, 384 or 1536 well plates (1,2). Furthermore, due to increased sample capacity, Bright-Glo™ Reagent provides greater assay sensitivity than Luciferase Assay Reagent in most applications (Figure 1).

The Bright-Glo™ and Steady-Glo™ Reagents are complementary in their characteristics based on the inverse relationship between luminescence duration and assay sensitivity (Figure 1; 1). Generally, as the half-life of luminescence increases, assay sensitivity decreases. The Steady-Glo™ Reagent, developed in 1998, provides very long luminescence duration, changing only about 10% per hour. This reagent is designed for circumstances in which many microplates are processed as a batch, as is common in high-throughput screening applications. However, to achieve long luminescence duration, the assay sensitivity must be reduced.

In contrast, the newly developed Bright-Glo™ Reagent provides high assay sensitivity with lower luminescence duration. This reagent is designed for general research applications, and for high-throughput screens using robotics for continuous sample processing.

Table 1. Characteristics of Promega's Luciferase Assay Reagents.

	Bright-Glo™ Reagent	Steady-Glo™ Reagent	Luciferase Assay Reagent
Format	NH or H	NH or H	NH
Process	Continuous	Batch	Bench scale
Number of Steps	1	1	4
Sensitivity	Highest	Lower	Higher
Signal Half-Life	~30 minutes	~ 5 hours	~12 minutes
Precision	High	High	High
Cell Lysis Time	~2 minutes maximum	~5 minutes maximum	NA
Reagent Prep Time	<30 seconds	<30 seconds	Up to 40 minutes

NH = nonhomogeneous; H = homogeneous; NA = not applicable.

Both the Bright-Glo™ and Steady-Glo™ Reagents provide the highest standards in assay quantitation, sensitivity and convenience. The choice between these reagents is determined by weighing the relative importance of assay sensitivity versus luminescence duration (Table 1). The reagents are based on the same underlying design principles, and thus may be used interchangeably, as experimental needs change. This can be particularly useful as research projects move through different phases, from early-stage discovery to high-throughput screening. By using the same reagent system in all phases, repeated characterizations and validations of cell lines and assay performance can be avoided.

The following describes in more detail how the Bright-Glo™ and Steady-Glo™ Reagents can be used in different research applications, including both manual and automated analysis of luciferase gene expression.

General research applications

The traditional method for measuring luciferase reporter expression requires removal of the culture medium and preparation of a cell lysate. Since this demands the separation of two components, the cells from the culture medium, the method is considered nonhomogeneous and is usually performed manually. Both the Bright-Glo™ and Steady-Glo™ Reagents support this method, although Bright-Glo™ Reagent is generally preferred, because relatively few samples are processed (sufficient to be contained in a single 96 well plate). The use of Bright-Glo™ Reagent allows the highest possible assay sensitivity, because long luminescence duration is not required.

E-mail *techserv@promega.com* for technical questions

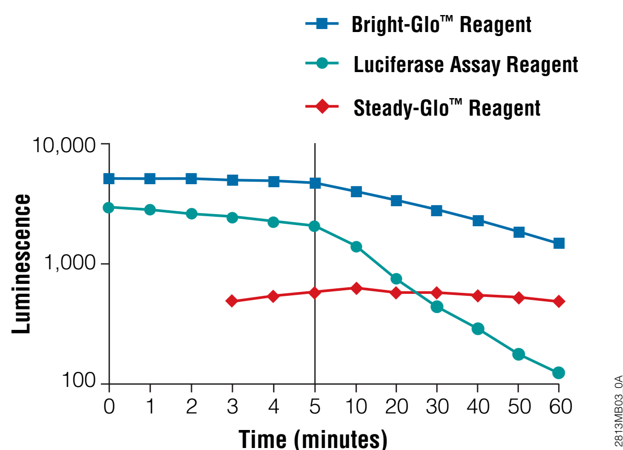


Figure 1. Luminescence profiles for Promega firefly luciferase assays. Purified luciferase (2.2×10^{-10}M with 1mg/ml BSA) was quantified in a 96 well plate according to the Technical Manual for each assay reagent. Luciferase was measured from 100µl of Glo Lysis Buffer for Steady-Glo™ and Bright-Glo™ Reagents (3,4). For Luciferase Assay Reagent, luciferase was measured from 20µl of Cell Culture Lysis Reagent (5). Each luminescent reaction was initiated by adding 100µl of the respective assay reagent. Traces represent repeated measurements of a single 96 well plate using a 1-second integration per well. Each data point is the average of 3 wells; relative standard error ≤3.3%.

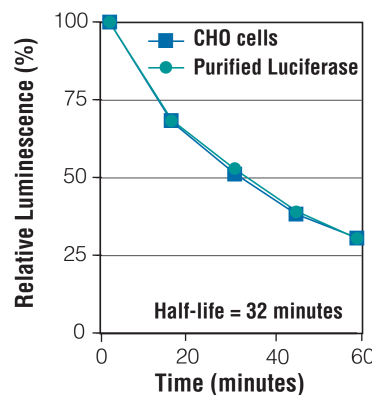
Note: For more information on Glo Lysis Buffer used in this study, please e-mail Promega's Tecnical Services Department (*techserv@promega.com*).

Luminescence from a reaction using Bright-Glo™ Reagent declines at a rate of approximately 10% per 5 minutes (i.e., a half-life of at least 24 minutes). This relatively short luminescence duration enables approximately a seven-fold increase in assay sensitivity over the Steady-Glo™ Reagent. The relatively short luminescence duration of Bright-Glo™ Reagent has little effect on assay precision, since 96 well plates are generally measured in less than 5 minutes (using measurement times of 1–2 seconds per well).

Luciferase expression is usually quantitated in terms of "relative luminescence," which is the measure of luminescence from an experimental sample relative to that of a control sample. Since both samples and controls are included in the same 96 well plate, they are always measured within minutes of each other. Thus, regardless of when the Bright-Glo™ Reagent is added to the plate, the relative luminescence between the experimental samples and controls will remain constant. However, we recommend that the plate be read within 15 minutes of adding the Bright-Glo™ Reagent to maximize assay sensitivity.

Assay precision can be further improved by averaging the measurements of replicate samples and replicate controls within the 96 well plate to reduce statistical variability. To minimize the effect of luminescence duration on assay precision, the samples and controls should be placed near each other, or the controls should be placed at both the beginning and end of the experimental samples. In this manner, the precision of relative luminescence should reveal <5% variability for the experimental samples.

If a more objective unit of quantitation than relative luminescence is desired, simply include control samples of purified firefly luciferase at known concentrations. This allows luciferase expression to be reported in terms of femtograms (10^{-15} grams) or attomoles (10^{-18} moles) of luciferase. Only a few concentrations of firefly luciferase are necessary to establish a reliable calibration curve, since the assay is strictly linear over seven decades of enzyme concentration.



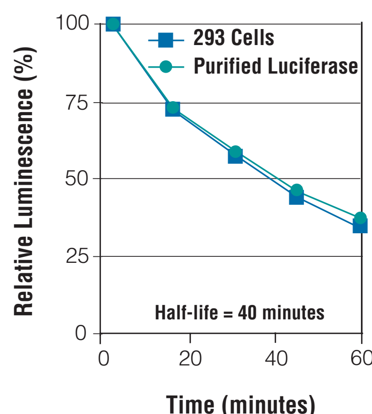
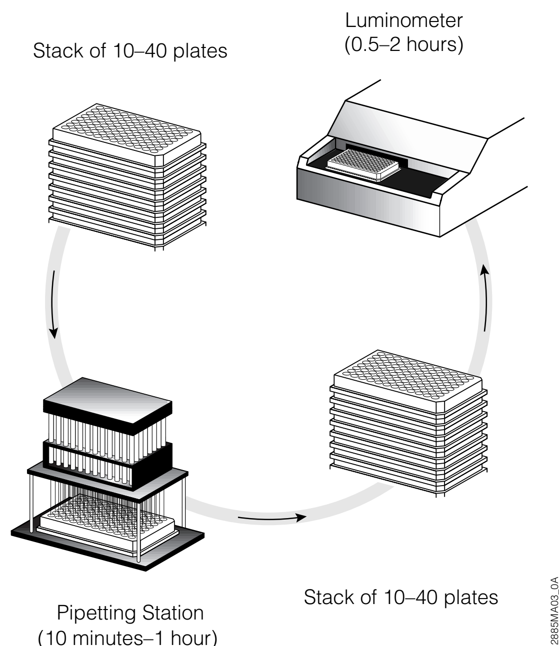


Figure 2. Reaction kinetics for purified firefly luciferase and luciferase expressed by stably transfected mammalian cells. Samples in 96 well plates consisted of either 100µl of purified enzyme (2.2×10^{-11}M with 1mg/ml BSA) or mammalian cells that had been stably transfected with the luciferase reporter gene. **Panel A:** CHO cells ($\sim 1 \times 10^4$ per well) in F12 medium. **Panel B:** 293 cells ($\sim 2.5 \times 10^4$ per well) in DMEM. Luminescence measurements were integrated over 1 second per well. Data show little difference in relative luminescence over time between the transfected cells and the purified enzyme in the same culture medium.

In the traditional nonhomogeneous assay method, cells are typically lysed in several hundred microliters of lysis buffer after removal of the culture medium. Aliquots of the cell lysates are transferred to a 96 well plate, where assay reagent can be added to multiple samples simultaneously using a multichannel pipettor. Using the standard reagent volume of 100µl, the capacity of the Luciferase Assay Reagent[a] allows assay of only 20µl aliquots of lysate. However, the capacity of the Bright-Glo™ Reagent allows assay of 100µl aliquots of lysate. This enables greater assay sensitivity for quantitation of gene expression. Even when measuring luminescence using individual assay tubes, this increased assay sensitivity may make Bright-Glo™ Reagent the preferred assay reagent.

Although the nonhomogeneous assay method is relatively easy to perform on a small number of samples, it becomes increasingly cumbersome with increasing sample numbers. In homogeneous assays, the assay reagent is



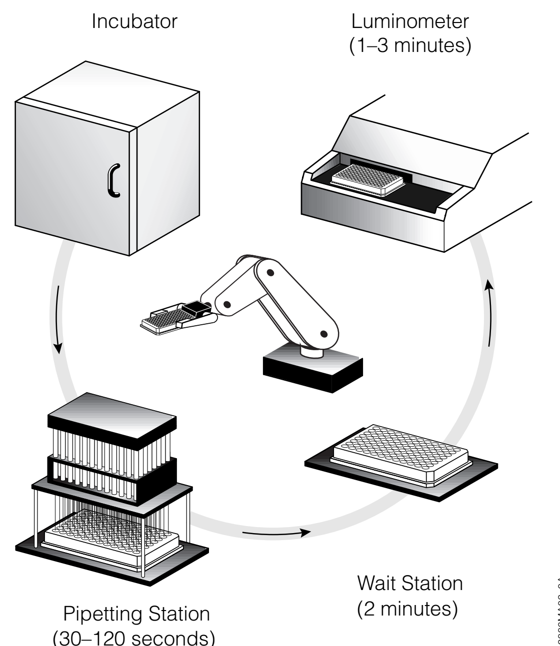


Figure 3. Schematics of batch and continuous processing of reporter assays. Panel A: The entire group of plates passes through each step in the process as a unit. All plates are removed from the incubator at the same time, reagent is dispensed into the plates and they are moved to the luminometer for measurement. **Panel B:** Each plate passes through each step in the process independently of the other plates. Multiple workstations may be used simultaneously, shortening the time required for each individual plate to complete the entire process.

added directly into the culture medium, thus avoiding the need to prepare lysates altogether. The assay reagent, when combined with the culture medium, causes cell lysis and supports the luminescence reaction. To avoid the need for subsequent sample transfer, cells are grown directly in the 96 well assay plates. At the time of assay, assay reagent is added to each well in a volume equal to the cell culture medium. The plate is then placed into the luminometer.

As with the nonhomogeneous assays, the Bright-Glo™ Reagent is generally preferred for maximum assay sensitivity when reading only one, or at most a few 96 well plates. To allow sufficient time for complete cell lysis, a delay of up to 2 minutes is recommended after addition of the Bright-Glo™ Reagent and prior to measuring luminescence. It is possible that some cell types may require more time for complete lysis.

Both the Bright-Glo™ and Steady-Glo™ Reagents were developed to be compatible with commonly used culture media for mammalian cells (e.g., DMEM, RPMI 1640, MEMα, F12) with up to 10% added calf or fetal bovine serum. Because half of the reaction volume is derived from culture media, the media composition can affect the luminescence duration and sensitivity. However, these effects are generally small. Furthermore, the relative luminescence between the experimental samples and controls should be unaffected since the same medium is used for both. The luminescence assayed from cells is almost indistinguishable from purified enzyme in the same medium (Figure 2).

Although Bright-Glo™ and Steady-Glo™ Reagents are broadly compatible with standard media compositions for cell culture, some specialized media may yield unexpected effects. To help researchers anticipate these, the assay reagents are supplied with technical manuals containing extensive appendices detailing assay performance under a wide range of conditions (3,4).

High-throughput screening

High-throughput screening relies on laboratory automation to process thousands to tens-of-thousands of samples per hour. Thus, in these applications, homogeneous assays are a higher priority, since separating sample components is relatively slow and requires more complex processing techniques. Furthermore, microplates with high sample densities, such as 384 and 1536 well plates, are commonly used to reduce sample size and increase throughput. Sample processing in these high-density plates is virtually impossible by manual techniques. The Bright-Glo™ and Steady-Glo™ Reagents have been designed for optimal performance with laboratory automation.

Automated screening applications work by processing stacks of microplates, each stack commonly containing 10–40 plates. Typically, each plate in the stack contains the same experimental controls. During the course of the screening experiment, samples in each plate are compared to controls on the same plate. This compensates for experimental errors that may occur due to the extended time required to process all plates in a stack. The stack may be processed by two methods, either batch or continuous process.

During batch processing, the entire stack of plates is operated upon by each step of the screen before performing the next step (Figure 3, Panel A). In the case of a luciferase assay, reagent is added to all of the plates before they are measured in a luminometer. This method is relatively simple and requires the least investment in laboratory automation. The only requirements are a plate stacker coupled to a pipetting workstation and a plate stacker coupled to a luminometer. The plate stack can be manually moved from one workstation to the next. Because of its simplicity, batch processing has been a popular method for high-throughput screening.

Several hours may be required to process the entire stack, so extended luminescence is necessary. Steady-Glo™ Reagent is preferred for these applications. Measurements of experimental samples and controls are always within minutes of one another since each plate is read relatively quickly, usually in about 1–3 minutes. Thus, precision of the relative luminescence remains high for each plate. The extended luminescence ensures that assay sensitivity remains high throughout the screen.

During continuous processing, each plate is processed independently of other plates in the stack (Figure 3, Panel B). For luciferase assays, each plate is processed in sequence by adding reagent and measuring in a luminometer. This method usually requires some form of transport robotics, since plates are continuously in transit from the pipetting workstation to the luminometer. Although requiring more complex automation, this method supports greater sample throughput, as all workstations can be utilized simultaneously, with the transport robot coordinating interactions between the workstations.

Continuous processing is particularly efficient for luciferase assays, since long luminescence duration is not required. This allows greater assay sensitivity, which in turn, reduces the time required for quantitation by the luminometer. Bright-Glo™ Reagent is preferred for these applications. Because the workstations are coupled through the transport robot, the overall throughput by continuous processing is limited by the slowest step. Usually, this is sample quantitation by the luminometer. This is especially true in ultrahigh-throughput applications (~100,000 samples per day) using a CCD luminometer to quantify all wells of 384 or 1536 well plates simultaneously. Although faster than a conventional PMT-based luminometer for high sample-density plates, the CCD luminometers are generally not as sensitive.

Bright-Glo™ Reagent can enable greater sample throughput in these applications due to its greater assay sensitivity. As described, measurements of the experimental samples and controls are always within minutes of one another since the plates are read relatively quickly. In a CCD luminometer, samples and controls are measured at the same time. Thus, the precision of the relative luminescence remains high regardless of the shortened luminescence duration. Assay sensitivity decreases more quickly than with the Steady-Glo™ Reagent, but remains high over the short time required for continuous processing. Assay sensitivity of Bright-Glo™ Reagent should exceed that of the Steady-Glo™ Reagent for about 1.5 hours after reagent addition (Figure 1).

Conclusions

Bright-Glo™ and Steady-Glo™ Reagents are complementary assay reagents designed to anticipate the needs of virtually any luciferase application. They are optimized for both manual and automated methodologies, and they support both nonhomogeneous and homogeneous assay formats. They are useful both in general research applications and high-throughput screening. They are broadly compatible with different luminometry equipment, including microplate luminometers based on PMT or CCD detectors. In all applications, they support high assay precision, sensitivity and convenience. The Bright-Glo™ Reagent in particular provides the highest assay sensitivity available for most applications. The versatility of these assay reagents allows for efficient technology transfer between different laboratories and project phases. For more information on Promega's bioluminescence products, see www.promega.com/biolum.

References

1. Hawkins, E., Jennens-Clough, M. and Wood, K.V. (1999) *Promega Notes* **70**, 7.
2. Wood, K.V. (2000) *Promega Notes* **74**, 3.
3. *Bright-Glo™ Luciferase Assay System Technical Manual* #TM052, Promega Corporation.
4. *Steady-Glo™ Luciferase Assay System Technical Manual* #TM051, Promega Corporation.
5. *Luciferase Assay Systems Technical Bulletin* #TB281, Promega Corporation.

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Ordering Information

Product	Size	Cat.#
Bright-Glo™ Luciferase Assay System	10ml	E2610
	100ml	E2620
	10 × 100ml	E2650
Steady-Glo™ Luciferase Assay System	10ml	E2510
	100ml	E2520
	10 × 100ml	E2550
Luciferase Assay System	100 assays	E1500
Luciferase Assay Reagent 10-pack	1,000 assays	E1501
Luciferase Assay System with Reporter Lysis Buffer[a]	100 assays	E4030
Luciferase 1000 Assay System[a]	1,000 assays	E4550

Bright-Glo and Steady-Glo are trademarks of Promega Corporation.

[a]U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289, 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. Certain applications of this product may require licenses from others.

[b]Patent Pending. U.S. Pat. No. 5,283,179, Australian Pat. No. 649289 and other patents. Certain applications of this product may require licenses from others.