TECHNICAL MANUAL

Bright-Glo™ Luciferase Assay System

Instructions for Use of Products
E2610, E2620 and E2650

Revised 9/15
TM052

Bright-Glo™ Luciferase Assay System

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

Ultrahigh-throughput quantitation of luciferase expression in mammalian cells requires highly sensitive reagents that can adapt to continuous-process robotic systems. The Bright-Glo™ Luciferase Assay System[a] is designed specifically to meet the needs of continuous-process systems by providing robust, homogeneous assay chemistry that achieves high assay sensitivity and approximately half-hour signal half-life without prior sample processing. These attributes also benefit scientists using fewer samples who still require high sensitivity and ease of use.

The provided Bright-Glo™ Luciferase Assay Buffer and Substrate are combined (see Section 3.B) to form Bright-Glo™ Reagent.

1. Description (continued)

Selected Citations using the Bright-Glo™ Luciferase Assay System

- Bridges, J.P. *et al.* (2005) Expression of a human surfactant protein C mutation associated with interstitial lung disease disrupts lung development in transgenic mice. *J. Biol. Chem.* **278**, 52739-46.

  A minimal promoter from the gene encoding BiP was cloned into the pGL3-Basic Vector. The resulting construct was transfected into HEK293 cells and 48 hours post-transfection, cell lysates were prepared using Glo Lysis Buffer. The Bright-Glo™ Luciferase Assay System then was used to assess levels of luciferase expression.

- Chanda, S.K. *et al.* (2003) Genome-scale functional profiling of the mammalian AP-1 signaling pathway. *Proc. Natl. Acad. Sci. USA* **100**, 12153-8.

  A human cDNA library of ~20,000 sequences was tested for putative modulators of the activator protein-1 (Ap-1) signal transduction pathway. The plasmid library was co-transfected with luciferase reporter plasmids containing AP-1, p53 or Epo response elements. Transfections were performed in 384-well plates using HEK293, HCT116 or HepG2 cells. After 48 hours, the Bright-Glo™ Luciferase Assay was used to determine the level of luciferase activity in the transfections.

For additional peer-reviewed articles that cite use of the Bright-Glo™ Luciferase Assay System, visit:
www.promega.com/citations/

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Bright-Glo™ Luciferase Assay System	**10ml**	**E2610**

Each system contains sufficient reagents to perform 100 assays of 100µl each. Includes:

- 10ml Bright-Glo™ Luciferase Assay Buffer
- 1 vial Bright-Glo™ Luciferase Assay Substrate (lyophilized)

PRODUCT	SIZE	CAT.#
Bright-Glo™ Luciferase Assay System	**100ml**	**E2620**

Each system contains sufficient reagents to perform 1,000 assays of 100µl each. Includes:

- 100ml Bright-Glo™ Luciferase Assay Buffer
- 1 vial Bright-Glo™ Luciferase Assay Substrate (lyophilized)

PRODUCT	SIZE	CAT.#
Bright-Glo™ Luciferase Assay System	**10 × 100ml**	**E2650**

Each system contains sufficient reagents to perform 10,000 assays of 100µl each. Includes:

- 10 × 100ml Bright-Glo™ Luciferase Assay Buffer
- 10 vials Bright-Glo™ Luciferase Assay Substrate (lyophilized)

Storage Conditions: Store the lyophilized Bright-Glo™ Substrate at –20°C. The substrate also may be stored at 4°C for up to two weeks. Store the Bright-Glo™ Buffer below 25°C. Storage at room temperature is recommended to prevent the need for temperature equilibration when the reagent is reconstituted. Use the reconstituted reagent on the day it is prepared, or store at –70°C for up to one month.

Note: Cat.# E2650 is provided in one box containing two trays: a 10-pack tray of Bright-Glo™ Substrate to be stored at –20°C, and a 10-pack tray of Bright-Glo™ Buffer to be stored unfrozen but below 25°C.

CAUTION: The lyophilized Bright-Glo™ Substrate contains dithiothreitol (DTT) and is therefore classified as hazardous. The reconstituted reagent is not known to present any hazards, as the concentration of DTT is less than 1%. However, we recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents. Promega assumes no liability for damage resulting from handling or contact with these products.

3. Performing the Bright-Glo™ Luciferase Assay

3.A. General Considerations

The Bright-Glo™ Luciferase Assay System is designed for use with the following culture media containing 0–10% serum: RPMI 1640, MEMα, DMEM and Ham's F12. The signal half-life under these conditions will generally exceed 25 minutes at 22°C and is independent of enzyme concentration where the luciferase concentration is $<10^{-9}$M. Other media/sera combinations also can be used, but experimental verification of assay performance is recommended in these cases (Section 5.B). The luminescence signal also can be affected by changes in temperature or the presence of phenol red and organic solvents (Section 5.B).

Additional information about the Bright-Glo™ Luciferase Assay System can be found in Section 5.

Because the luminescent signal is affected by assay conditions, results should be compared only between samples measured using the same medium/serum combination. For analysis of multiple plates, the greatest accuracy can be obtained by incorporating a common control sample in each plate. By this method, luminescence measurements of each plate can be normalized to the control contained within the same plate. This allows the correction of small variations in luminescence that can occur over time or due to other variables such as temperature.

As some time is required for complete cell lysis, the Bright-Glo™ Luciferase Assay Reagent should be added to plates at least 2 minutes prior to quantifying luminescence. Complete cell lysis has been measured within 2 minutes of reagent addition to HeLa, CHO and NIH/3T3 cells. For maximal light intensity, samples should be measured within 15 minutes of reagent addition. The Bright-Glo™ Reagent is not designed for use with the automated reagent injectors that are integrated into some luminometers.

The Bright-Glo™ Luciferase Assay Reagent is not designed for use with the automated reagent injectors integrated into some luminometers.

3.A. General Considerations (continued)

To achieve linear assay performance at low light levels, background luminescence must be subtracted from all readings. No background is produced by the Bright-Glo™ Reagent or by mammalian cells lacking the luciferase gene, but background luminescence is a characteristic of luminometer performance. Some instruments also require verification of linear response at high light levels (consult the instrument manual).

Approximate stability of Bright-Glo™ Reagent after reconstitution: 10% loss of luminescence per 5 hours at room temperature, 10% loss per 24 hours at 4°C, and <5% loss after one month at –70°C. The reagent may be subjected to up to seven freeze-thaw cycles with no effect on potency.

3.B. Reagent Preparation

Transfer the contents of one bottle of Bright-Glo™ Buffer to one bottle of Bright-Glo™ Substrate. Mix by inversion until the substrate is thoroughly dissolved.

Notes:

1. Since luciferase activity is temperature-dependent, the temperature of the Bright-Glo™ Reagent should be held constant while quantitating luminescence. This is achieved most easily by using reagent equilibrated to room temperature, which is near the temperature optimum of luciferase (Section 5.B). Equilibration of the reagent prior to use is unnecessary when the buffer is stored at room temperature.
2. If the reagent is stored frozen after reconstitution, it must be thawed at temperatures below 25°C to ensure performance. Mix well after thawing. The most convenient and effective method to thaw or temperature equilibrate cold reagent is to place it in a water bath at room temperature.
3. For maximum reproducibility, equilibrate cultured cells to room temperature before adding the reagent.

3.C. Assay Procedure

1. Remove multiwell plates containing mammalian cells from the incubator. The plates must be compatible with luminescence measurements in the luminometer being used.
2. Add a volume of reagent equal to that of the culture medium in each well, and mix. For 96-well plates, typically 100µl of reagent is added to cells grown in 100µl of medium. For 384-well plates, typically 30µl of reagent is added to cells grown in 30µl of medium.
3. Wait at least 2 minutes to allow complete cell lysis, and measure in a luminometer (consult the instrument manual).

   Note: If the reagent is added to prelysed cells, luminescence may be measured immediately after reagent addition.

4. Related Products

Luciferase Assay Systems

Product	Size	Cat.#
Steady-Glo® Luciferase Assay System	10ml	E2510
	100ml	E2520
	10 × 100ml	E2550
Dual-Glo™ Luciferase Assay System	10ml	E2920
	100ml	E2940
	10 × 100ml	E2980
Dual-Luciferase® Reporter Assay System	100 assays	E1910
Dual-Luciferase® Reporter Assay System 10-Pack	1,000 assays	E1960
Dual-Luciferase® Reporter 1000 Assay System	1,000 assays	E1980
Luciferase Assay System	100 assays	E1500
Luciferase Assay System with Reporter Lysis Buffer	100 assays	E4030
Luciferase Assay Reagent, 10-Pack	1,000 assays	E1501
Luciferase Assay System Freezer Pack	1,000 assays	E4530
Luciferase 1000 Assay System	1,000 assays	E4550
Luciferase Assay Reagent	1,000 assays	E1483

Firefly Luciferase Reporter Vectors

Product	Size	Cat.#
pGL4.10[*luc2*] Vector	20µg	E6651
pGL4.11[*luc2P*] Vector	20µg	E6661
pGL4.12[*luc2CP*] Vector	20µg	E6671
pGL4.13[*luc2*/SV40] Vector	20µg	E6681

4. Related Products (continued)

Miscellaneous Luciferase Products

Product	Size	Cat.#
QuantiLum® Recombinant Luciferase	1mg	E1701
	5mg	E1702
Glo Lysis Buffer	100ml	E2661

Plasmid DNA Purification System

Product	Size	Cat.#
PureYield™ Plasmid Midiprep System	25 preps	A2492
	100 preps	A2495
PureYield™ Plasmid Maxiprep System	10 preps	A2392
	25 preps	A2393

5. Appendix

5.A. Overview of the Bright-Glo™ Luciferase Assay System

Transcriptional regulation, coupled to the expression of a reporter gene, is routinely used to study a wide range of physiological events. A common example is the analysis of receptor function by quantifying the action of specific receptor response elements on gene expression. Other examples include the study of signal transduction, transcription factors, protein-protein interactions, and viral infection and propagation (1,2). Events downstream of transcription, such as mRNA processing and protein folding, also can be analyzed.

Luciferase is a popular choice as a reporter for these applications because functional enzyme is created immediately upon translation, and the assay is rapid, reliable and easy to perform (3,4). Furthermore, analysis using luciferase as the genetic reporter is well suited to laboratory automation and high-throughput applications. For these reasons, luciferase is widely used in the biotechnology and pharmaceutical industries.

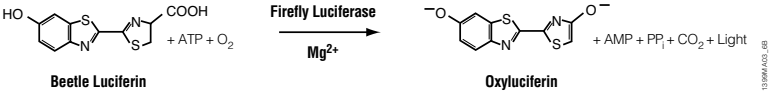

Figure 1. The luciferase reaction. Mono-oxygenation of luciferin is catalyzed by luciferase in the presence of Mg^{2+}, ATP and molecular oxygen.

Firefly Luciferase

Firefly luciferase is a 61kDa monomer that catalyzes the mono-oxygenation of beetle luciferin (Figure 1). Beetle luciferin is a relatively stable molecule found only in luminous beetles (which includes fireflies). The enzyme uses ATP as a cofactor, although most of the energy for photon production comes from molecular oxygen. The quantum yield is about 0.9, the highest of any known luminescent reaction (5). The gene encoding firefly luciferase (*luc*) is a cDNA clone that has been incorporated into a number of reporter vectors (Section 4).

Development of the Assay

Promega has developed a complete product line of high-quality reagents to quantitate firefly luciferase for the various needs of researchers. For general research applications requiring maximum sensitivity, Promega provides Luciferase Assay System (Cat.# E1500). This reagent differs from previous assay reagents in that it contains Coenzyme A to provide a more stable luminescence signal; however, this reagent requires complete control of the assay environment. Cell lysis must be preceded by removal of cell culture medium, which is generally not practical for high-throughput applications using robotic systems.

Promega has introduced the Steady-Glo® Luciferase Assay System (Cat.# E2520) for researchers who batch-process multiwell plates for high-throughput analysis. This homogeneous reagent generates luminescent signals with a half-life exceeding 5 hours in common culture media. However, the stability of the luminescent signal produced by the Steady-Glo® Reagent is accompanied by lower sensitivity of the reagent.

The Bright-Glo™ Luciferase Assay System was developed specifically to maximize assay sensitivity while providing a signal half-life generally exceeding 25 minutes. With this half-life, luminescence decreases by about 10% within 5 minutes, or the maximal time required to complete measurement of a 96-well plate when capturing data for 1 second per well. Like the Steady-Glo® System, the Bright-Glo™ Reagent is compatible with most commonly used culture media for mammalian cells (RPMI 1640, MEMα, DMEM and F12, with or without added serum) and can tolerate phenol red and organic solvents (Section 5.B). Furthermore, the reagent is tolerant of incomplete mixing (see next section, Assay Precision), making it suitable for multiwell plates. These performance criteria were achieved while maximizing luminescence intensity to achieve the highest possible assay sensitivity. Moreover, mammalian cells have little effect on assay performance compared with purified luciferase diluted into the same culture medium (Figure 2).

5.A. Overview of the Bright-Glo™ Luciferase Assay System (continued)

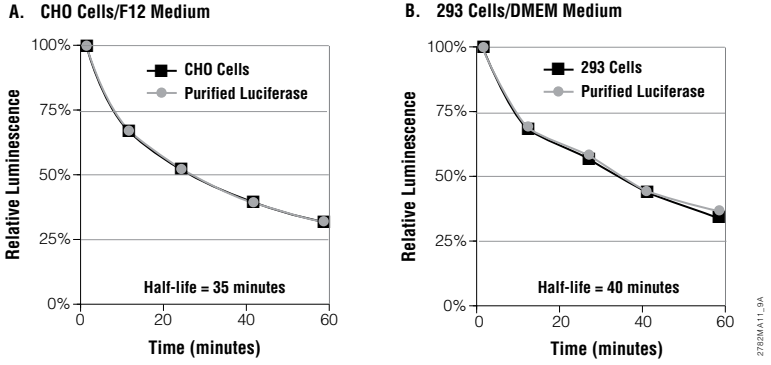

Figure 2. Reaction kinetics for purified firefly luciferase and luciferase expressed by stably transfected mammalian cells. Samples in 96-well plates consisted of either 100µl of purified enzyme (2.2×10^{-11}M with 1mg/ml BSA) or mammalian cells that are stably transfected with the luciferase reporter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

All Promega luciferase reagents were developed to achieve maximum efficiency and reproducibility. Because temperature is an important factor in assay performance (Section 5.B), one important characteristic is the stability of the Bright-Glo™ Luciferase Assay Buffer at room temperature. Pre-equilibrating the buffer by storage at room temperature eliminates the need to temperature equilibrate the reconstituted reagent. This makes assay reproducibility easier to achieve. Other physical properties, such as density, viscosity and foaming activity, also were optimized for maximum assay reproducibility and compatibility with standard laboratory equipment.

Assay Precision

The Bright-Glo™ Luciferase Assay, like other Promega extended-half-life luciferase assays, provides greater assay precision through a novel formulation, making it more tolerant of variations in reactant concentrations (Figure 3). Because of this tolerance, deviations in luminescence between sample wells caused by incomplete mixing, pipetting errors or sample evaporation are minimized. This is particularly important for high-throughput processing of multiwell plates, where accurate pipetting and sample mixing may be difficult to achieve.

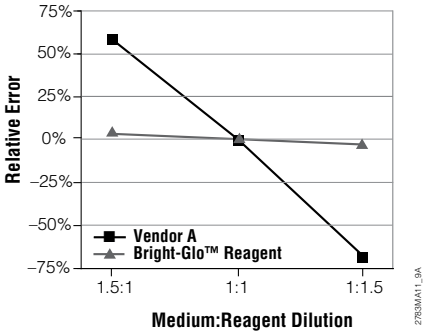

Figure 3. Effects of reagent concentration on assay precision. Purified firefly luciferase (2.2×10^{-10}M in RPMI 1640 with 1mg/ml BSA) was added to a 96-well plate at 100µl per well. Either Bright-Glo™ Reagent or a conventional extended-half-life reagent then were added to create the indicated dilutions. Luminescence measurements were integrated over 1 second per well. Relative error was calculated as the percent change of the luminescence from that produced by the 1:1 dilution sample. Number of samples = 3; relative standard error ≤2.6%.

Luciferase assays that are designed for maximum sensitivity (i.e., maximum luminescence intensity) generate luminescence signals with half-lives of approximately 12–15 minutes. The decay of luminescence in these samples is too fast to measure the luminescence of an entire multiwell plate without a significant drop in signal between the first and last well on the plate. Although the details are not well understood, this decay is due to slow degradation of the enzyme during catalysis. To reduce the rate of degradation, thereby providing a more stable luminescent signal, the rate of catalysis also must be reduced. Hence, steady-state luciferase assays for high-throughput have lower luminescence intensities, resulting in lower assay sensitivities. The Bright-Glo™ Luciferase Assay, however, with its shorter half-life compared to other extended-half-life assays, provides many times more light than the Steady-Glo® Reagent or other extended-half-life assays.

Even slight inhibition of the rate of catalysis may cause the assay to become susceptible to variations in reagent concentration. Specifically, enzyme activity (and light output) may be too low if the inhibitor concentration is too high. Conversely, activity may be too high if the inhibitor concentration is too low, leading to rapid decay of the enzyme and lower signal stability. This behavior is evident in conventional extended-lifetime luciferase assay reagents (Figure 3) and can reduce experimental precision under standard laboratory conditions.

The formulation of the Bright-Glo™ Reagent largely overcomes the concentration effect of enzyme inhibition. Luminescence intensity of the assay is little changed over a wide range of reagent concentrations, making the reagent less sensitive to pipetting and mixing conditions. This characteristic allows good precision to be more easily achieved with automated liquid-handling equipment, potentially reducing the occurrence of false positive or false negative results.

5.B. Conditions Affecting Assay Performance

The data presented in this section are intended to provide a general overview of assay characteristics under a wide range of experimental conditions. Note that the Bright-Glo™ Luciferase Assay System is chemically different from other luciferase assays designed for high-throughput analysis. As a result, the data presented here may not be applicable to other luciferase assay systems.

Purified luciferase diluted in culture medium was used to generate the data presented in this section. This was done to illustrate performance characteristics of the reagent while avoiding experimental complexities common to cell culture. However, as demonstrated in Figure 2, purified luciferase diluted in culture medium shows little or no difference from enzyme expressed in transfected cells. BSA (1mg/ml) was added to the wells to simulate protein that would normally be contributed by the cells. However, addition of BSA is not required when using the Bright-Glo™ Reagent.

Culture Medium

In the Bright-Glo™ Assay, culture medium and any other compounds contained therein comprise half of the chemical environment that defines the luminescent reaction. Although the Bright-Glo™ Reagent is designed to work in conjunction with many common culture media, compositional differences between these different media can affect assay characteristics (e.g., light intensity and signal stability).

The Bright-Glo™ Luciferase Assay System is designed to provide relatively high luminescence with signal half-lives generally exceeding 25 minutes when using common growth media. However, performance differences are evident between these media (Figure 4), as well as between different manufacturers of the same media. Sometimes, differences are also evident between lots of media from the same manufacturer. Although these differences are generally small and do not diminish the utility of the Bright-Glo™ Assay, controls should be incorporated into every batch of plates to correct for this variability.

Serum

Bright-Glo™ Luciferase Assay System is compatible with serum in medium. The Bright-Glo™ Reagent is designed to be used with serum concentrations from 0–10%, and the luminescence signals generated are minimally affected by the presence of fetal bovine or calf sera (Figure 5).

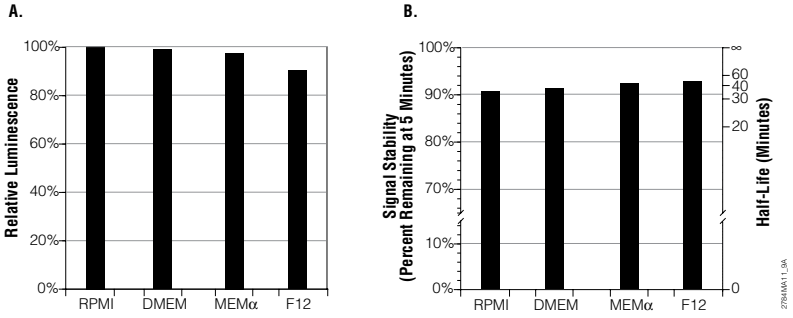

Figure 4. Relative intensity and signal stability of firefly luciferase in four common media. Purified firefly luciferase (2.2×10^{-10}M in medium with 1mg/ml BSA) was added to a 96-well plate at 100µl per well. Dilutions were made in RPMI 1640, DMEM, MEMα or F12 medium. Bright-Glo™ Reagent was added, and luminescence measurements were integrated over 1 second per well. **Panel A.** Luminescence is shown relative to the light output generated in RPMI 1640. **Panel B.** Signal stability in different media expressed as percent remaining per hour and as half-life. Number of samples = 3; relative standard error ≤1.2%.

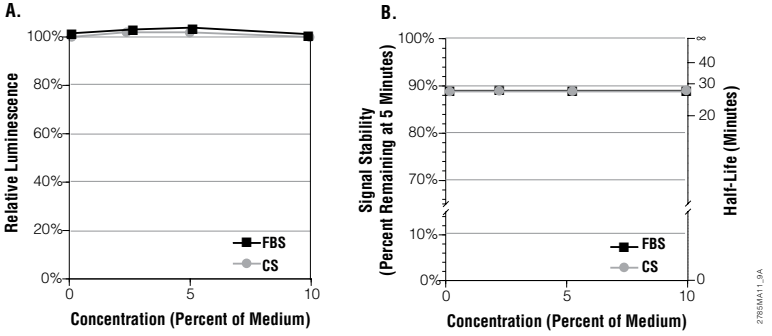

Figure 5. Effects of sera on intensity and signal stability. Purified firefly luciferase (2.2×10^{-10}M in medium with 1mg/ml BSA) was added to a 96-well plate at 100µl per well. DMEM containing various concentrations of fetal bovine serum (FBS) or calf serum (CS) was used for the assay. Bright-Glo™ Reagent was added, and luminescence measurements were integrated over 1 second per well. **Panel A.** Luminescence is shown relative to that measured without serum. **Panel B.** Signal stability at various concentrations of sera expressed as percent remaining per hour and as half-life. Number of samples = 3; relative standard error ≤1.7%.

5.B. Conditions Affecting Assay Performance (continued)

Buffered Saline

Although luminescence measurements are generally performed in the culture medium in which the cells were grown, sometimes conditions require that the assay be performed using buffered saline (10mM buffer [pH 7.2–7.4], 150mM NaCl) or that the assay be performed with prelysed cells in buffered saline. As illustrated in Table 1, different buffers produce luminescent reactions with varying light intensities and signal stabilities.

Table 1. Luminescence Measured in Buffered Saline Solutions.

Buffer/Medium	% Relative Luminescence	% Relative Standard Error	% Remaining After 5 Minutes
RPMI 1640	100	0.8	93
Phosphate (Dulbecco's PBS)	87	0.5	92
HEPES	92	1.0	92
Glo Lysis Buffer	111	0.6	93

Purified firefly luciferase (2.2×10^{-10}M with 1mg/ml BSA) was added to a 96-well plate at 100µl per well. Dilutions were made in RPMI 1640 medium, Dulbecco's PBS, Glo Lysis Buffer (GLB; Cat.# E2661) or 10mM HEPES (pH 7.4) with 150mM NaCl. Bright-Glo™ Reagent was added, and luminescence measurements were integrated over 1 second per well. Luminescence is shown relative to that measured in RPMI 1640. Number of samples = 3.

Phenol Red

Phenol red is a widely used pH indicator that is added to cell culture media. Many commercial medium formulations contain 5–15mg/L phenol red, evident by a red coloration in the medium. This compound will reduce assay sensitivity (Figure 6). However, in most applications, the presence of phenol red will not significantly affect the utility of the Bright-Glo™ Assay. To minimize its effect, use as little phenol red as possible in the culture medium.

Organic Solvents

Organic solvents are typically present in the luciferase assay, since they are used to stabilize and solubilize screening compounds. DMSO, isopropanol and ethanol have little effect on the assay (Figure 7). The compatibility of other solvents should be verified prior to use.

Medium Compositions

The Bright-Glo™ Reagent is designed to work with most commonly used culture media for mammalian cells. However, under some circumstances it may be desirable to use alternative media formulations or cell lysates containing buffered saline. Although it is difficult to predict precisely how these alternative formulations might affect performance of the Bright-Glo™ Luciferase Assay System, the data below can be used as a guideline (Figure 8).

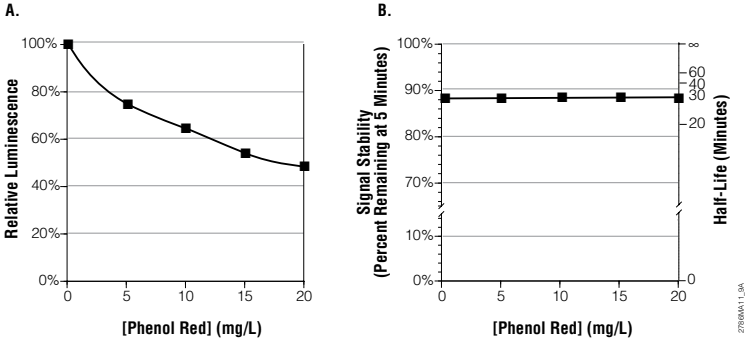

Figure 6. Effect of phenol red on light intensity and signal stability. Purified firefly luciferase (2.2×10^{-10}M in medium with 1mg/ml BSA) was added to a 96-well plate at 100µl per well. DMEM containing various concentrations of phenol red was used for the assay. Bright-Glo™ Reagent was added, and luminescence measurements were integrated over 1 second per well. **Panel A.** Luminescence is shown relative to that measured without phenol red. **Panel B.** Signal stability at various concentrations of phenol red expressed as percent remaining per hour and as half-life. Number of samples = 3; relative standard error ≤0.7%.

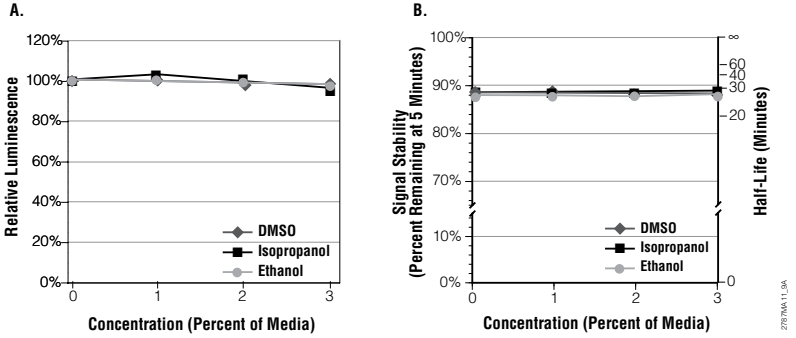

Figure 7. Effect of organic solvents on luminescence intensity and signal stability. Purified firefly luciferase (2.2×10^{-10}M in medium with 1mg/ml BSA) was added to a 96-well plate at 100µl per well. DMEM containing various concentrations of DMSO, isopropanol or ethanol was used for the assay. Bright-Glo™ Assay Reagent was added, and luminescence measurements were integrated over 1 second per well. **Panel A.** Luminescence is shown relative to that measured without organic solvents. **Panel B.** Signal stability at various concentrations of solvent expressed as percent remaining per hour and as half-life. Number of samples = 3; relative standard error ≤1.9%.

5.B. Conditions Affecting Assay Performance (continued)

Figure 8 shows the effect of different concentrations of several components commonly used to define medium or buffered saline. Per graph, the concentration of each component is indicated for RPMI 1640, DMEM, MEMα and F12 media.

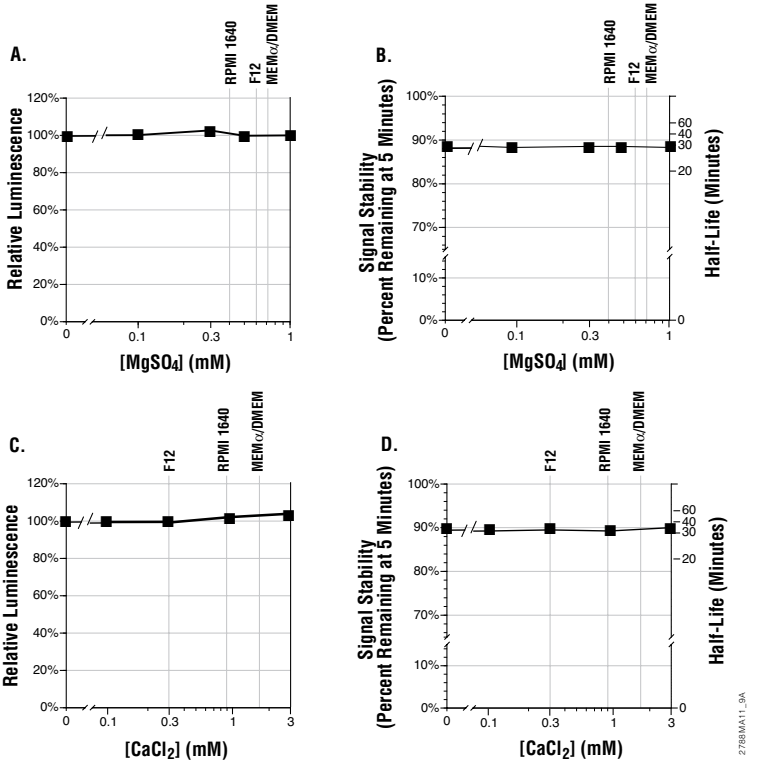

Figure 8. Effect of medium compositions on luminescence intensity and signal stability. Purified firefly luciferase (2.2×10^{-10}M in medium with 1mg/ml BSA) was added to a 96-well plate at 100µl per well. Each component (indicated on X axis) was tested in Dulbecco's PBS. In those cases where the component is also a part of the buffered saline (i.e., NaCl), the composition of the buffered saline was adjusted to yield the desired concentration range of the component being tested. For the phosphate titration, 10mM HEPES was used to buffer the solution at pH 7.4. Luminescence measurements were integrated over 1 second per well. **Left Panels.** Luminescence is shown relative to luminescence in the absence of the component. **Right Panels.** Signal stability at various concentrations of each component expressed as percent remaining per hour and as half-life. At least three samples were used for each data point, and all relative standard errors were <1.8%.

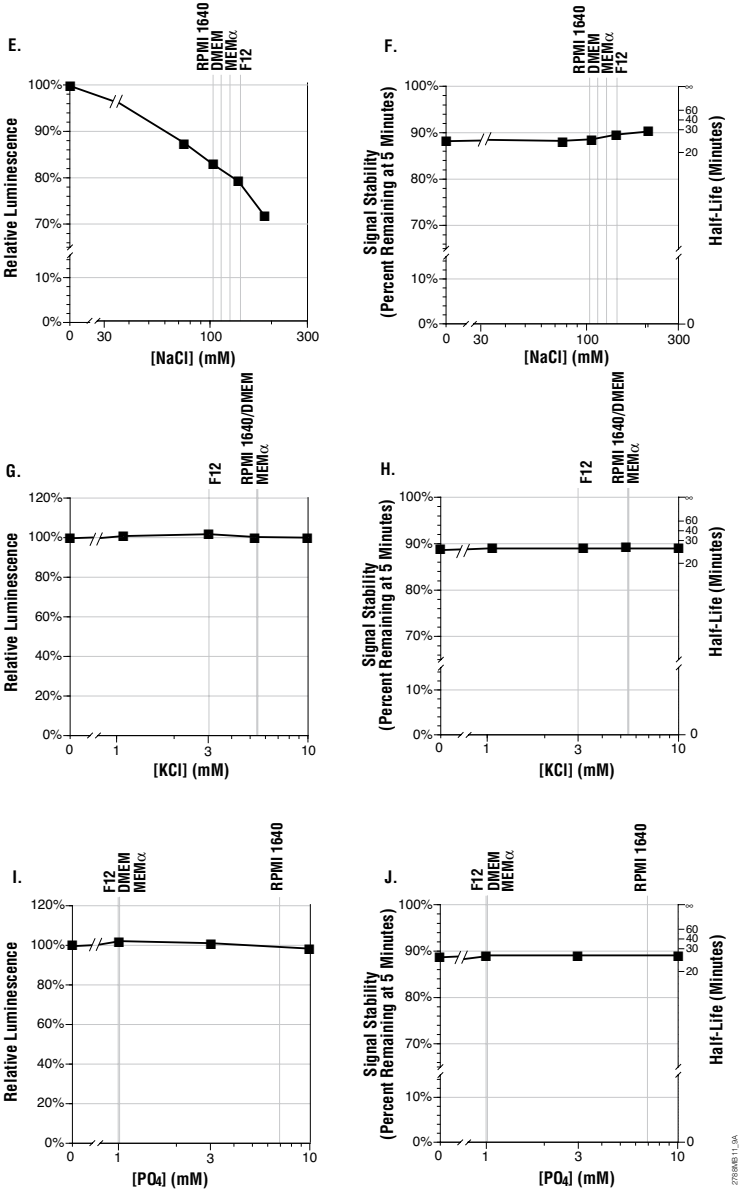

Figure 8. Effect of medium compositions on luminescence intensity and signal stability (continued).

5.B. Conditions Affecting Assay Performance (continued)

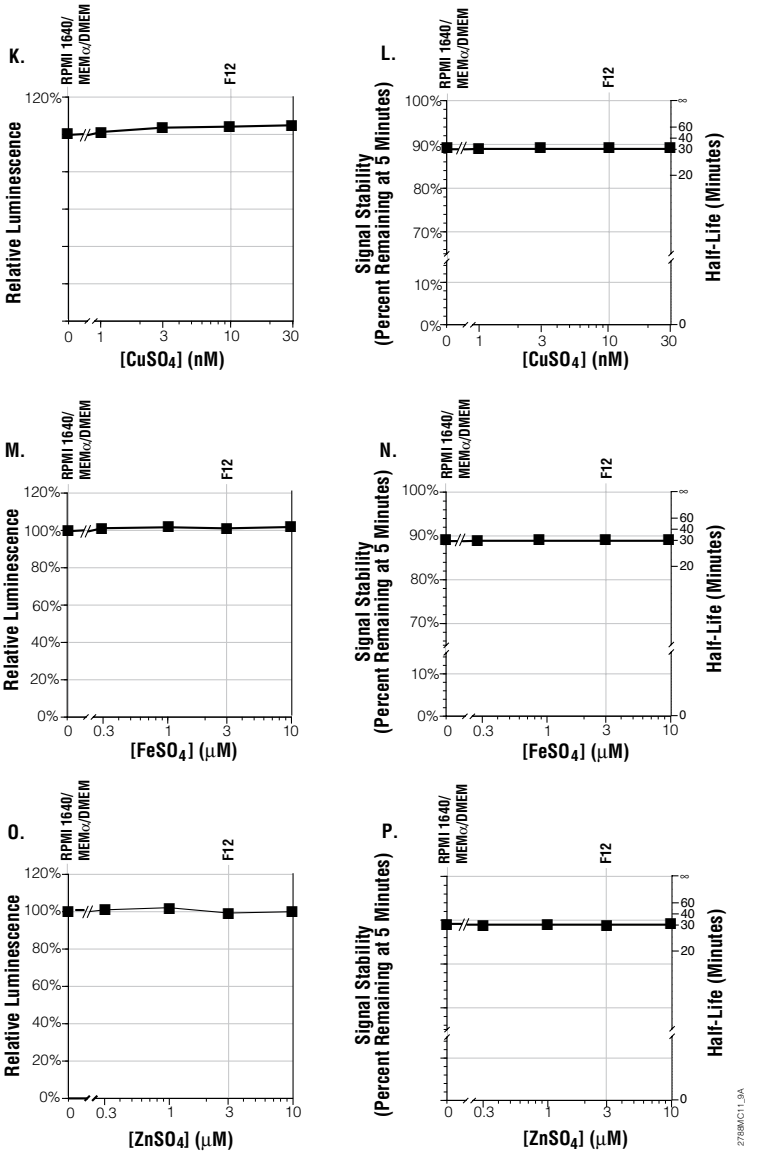

Figure 8. Effect of medium compositions on luminescence intensity and signal stability (continued).

Temperature

Since luciferase activity is temperature-dependent, temperature is an important factor in experimental precision (Figure 9). Good precision can be achieved most easily by performing all experiments at room temperature, as this is near the temperature optimum for luciferase activity. Thus, the assay reagent should be at room temperature before beginning measurements.

As mentioned previously, Bright-Glo™ Buffer can be stored at room temperature to avoid the need to temperature equilibrate the reagent before use. The heat capacity of the dried substrates is low; therefore, reconstitution of Bright-Glo™ Substrate with room-temperature Bright-Glo™ Buffer yields a Bright-Glo™ Assay Reagent that is ready for use. If temperature equilibration is needed, use a water bath at room temperature for maximum heat transfer. Do not use a water bath above 25°C.

Lower temperatures result in increased signal stability, but luminescent intensity is lowered. If cold reagent is used, luminescence will slowly increase during the experiment as the reagent warms. Higher temperatures cause an increase in luminescence, but the signal becomes less stable. This can occur if the culture plates are too warm or if the luminometer produces excess heat within the reading chamber.

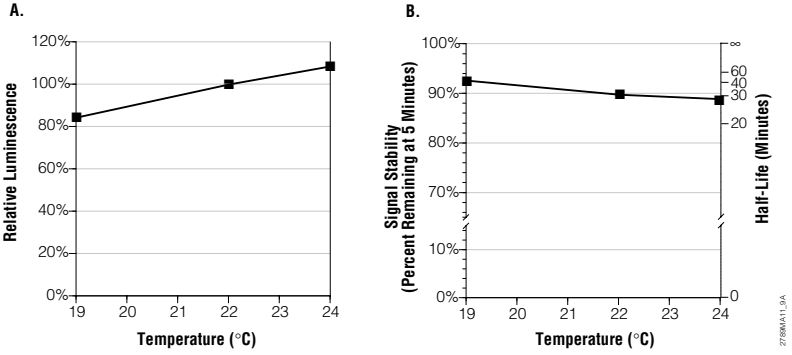

Figure 9. Effect of temperature on luciferase luminescence. Purified firefly luciferase (2.2×10^{-10}M in DMEM with 1mg/ml BSA) was added to luminometer tubes at 100µl per sample. Bright-Glo™ Reagent was added, and reactions were stored at various temperatures. Luminescence was measured every 15 minutes for 1.5 hours (integrated over 1 second per sample). **Panel A.** Luminescence is shown relative to that measured at 22°C. **Panel B.** Signal stability at various temperatures expressed as percent remaining per hour and as half-life. Number of samples = 3; relative standard error ≤3.1%.

5.B. Conditions Affecting Assay Performance (continued)

Some researchers may choose to use Bright-Glo™ Reagent as a nonhomogeneous reagent to measure luciferase activity for previously lysed cells containing luciferase. Temperature control is critical for this application, and lysates should be at room temperature before beginning the luminescence reaction. The large volume of lysate within the Bright-Glo™ Assay reaction (one half of the reaction volume) necessitates temperature equilibration of the sample for maximum luminescence (Figure 10).

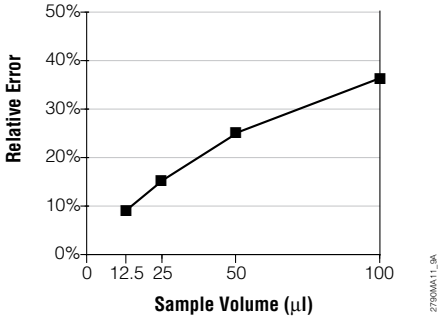

Figure 10. Effect of iced samples on luminescence. Purified firefly luciferase (2.2×10^{-10}M in DMEM with 1mg/ml BSA) that was equilibrated to room temperature (~22°C) or on ice was added to luminometer tubes at various sample volumes. Bright-Glo™ Reagent (100µl) was added to each sample, and the luminescence was measured. Error is the relative decrease in luminescence measured for each sample on ice compared to the same volume of sample at room temperature. Number of samples = 3; relative standard error ≤3.5% (each point).

Reagent Mixing

As described in Section 5.A, the Bright-Glo™ Reagent is designed to minimize the effect of concentration differences that can result from incomplete sample mixing. As with other performance criteria, this is affected by the composition of the culture medium (Figure 11). However, in all cases, the relative error caused by variable reagent concentration is much less than that of conventional extended-lifetime luciferase reagents (Figure 3).

Even though the effects of reagent concentration are minimized, good assay precision still depends on the efficiency of sample mixing. To produce the most uniform and reproducible data, the reagent and culture media should be completely mixed prior to measurement. Where operational constraints make this impractical, optimization of sample-handling procedures can significantly affect data quality. This is particularly important for 384- and 1536-well plates because of the small size of sample wells.

The most important handling consideration is the force and angle of reagent delivery into the wells of the plate. This can vary considerably depending on the brand and configuration of the pipette. Where reagent delivery is inefficient, adjusting the volume of reagent delivered or extending the incubation time before measurement can improve the results of the assay, as long as procedures are consistent and proper controls are included.

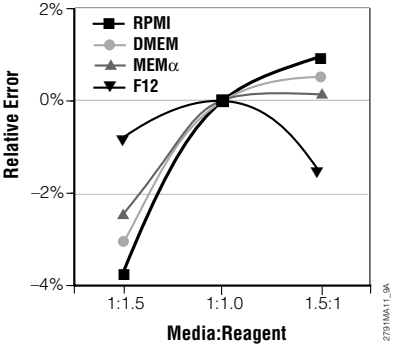

Figure 11. Effects of reagent concentration on assay precision. Purified firefly luciferase (2.2×10^{-10}M with 1mg/ml BSA) was added to a 96-well plate at 100µl per well. Bright-Glo™ Reagent was added, and luminescence measurements at various dilutions of each assay reagent were integrated over 1 second per well for each medium. Relative error was calculated as the percent change of the luminescence from that produced by the 1:1 dilution sample. Number of samples = 3; relative standard error (per data point) ≤1.2%.

5.C. Procedure for Use With Glo Lysis Buffer

Glo Lysis Buffer, 1X, is a proprietary formulation that promotes rapid lysis (within 5 minutes) of cultured mammalian cells without the need for scraping or performing freeze-thaws of adherent cells. Glo Lysis Buffer is fully compatible with Bright-Glo™ Assay Reagent to analyze firefly luciferase expression. Additionally, Glo Lysis Buffer is recommended as a diluent for firefly luciferase because it provides enzyme stability of at least 2 days at room temperature (Figure 12). Similarly, in studies at Promega, firefly luciferase expressed in CHO, HEK293 and HeLa cells is stable after 48 hours at 22°C.

5.C. Procedure for Use With Glo Lysis Buffer (continued)

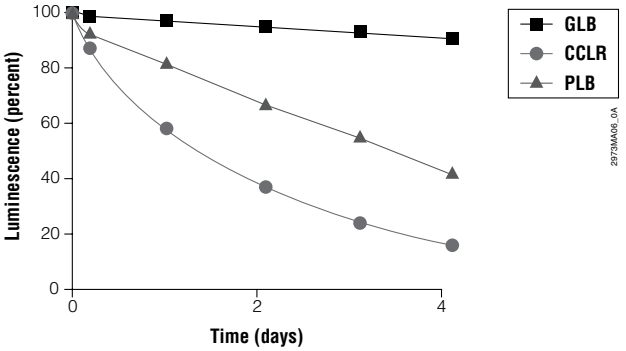

Figure 12. Stability of firefly luciferase in Glo Lysis Buffer. Purified firefly luciferase (2.2×10^{-10}M with 1mg/ml BSA) was added to Glo Lysis Buffer (GLB), Cell Culture Lysis Reagent (CCLR) or Passive Lysis Buffer (PLB). Luciferase was incubated at 22°C for up to 100 hours, with samples taken at 24-hour intervals and stored at –70°C. After 100 hours, all samples were thawed and initial luminescence measured. Luminescent reactions were initiated by adding 100µl of the respective lysis reagent to Bright-Glo™ Assay Reagent. Stability was determined as the amount of time necessary for initial luminescence to decrease to 90% of the luminescence at time zero.

Protocol

Glo Lysis Buffer can be used in a 1:1 ratio with reconstituted Bright-Glo™ Assay Reagent following the protocol below.

1. Equilibrate the Glo Lysis Buffer to 22°C before use.
2. Equilibrate cells to room temperature. Aspirate medium from the cells. Gently rinse with 1X PBS (rinse is optional).
3. Add a sufficient volume of Glo Lysis Buffer to the sample well or plate to cover cells (see Table 2). When using lysate for other applications, consider the volume needed for that application.

Table 2. Amount of Glo Lysis Buffer for Use With Various Plate Sizes.

Plate	Glo Lysis Buffer
100mm	3ml
60mm	1.1ml
35mm	500µl
6 wells	500µl
12 wells	200µl
24 wells	100µl
96 wells	100µl

4. Rock the plate slowly several times to ensure complete coverage of the cells with Glo Lysis Buffer.
5. Incubate for 5 minutes at room temperature to allow cell lysis.
6. Transfer the lysate to luminometer tubes, plate wells or vials, and add a volume of Bright-Glo™ Assay Reagent equal to the volume of Glo Lysis Buffer added. Measure luminescence with a luminometer, following the manufacturer's instructions.

   Note: Lysate prepared with Glo Lysis Buffer can be stored at –20°C or –70°C and is stable over several freeze-thaw cycles.

Firefly Luciferase Enzyme Dilution Protocol with Glo Lysis Buffer

To prepare a positive control using QuantiLum® Recombinant Luciferase (Cat.# E1701) dilute the enzyme 10^{-7} in 1X Glo Lysis Buffer containing 1mg/ml BSA. Equilibrate the luciferase to room temperature for 20 minutes before performing an assay. Alternatively, the QuantiLum® Recombinant Luciferase can be diluted to 10^{-6} in 1X Glo Lysis Buffer containing 1mg/ml BSA, then diluted 1:10 in cell culture medium.

Note: Enzyme dilutions can be prepared and stored for up to 3 months at –80°C. Prepare enzyme by diluting in Glo Lysis Buffer to 10^{-3} to 10^{-6}. Store in 500µl aliquots.

To perform a firefly luciferase activity assay, add 100µl of QuantiLum® Recombinant Luciferase, diluted as indicated above, to 100µl of Bright-Glo™ Assay Reagent. Read the sample in a luminometer following the manufacturer's instructions.

5.D. References

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5.E. Summary of Changes

The following changes were made to the 9/15 version of this document:

Expired patent information was removed.

[a]Certain applications of this product may require licenses from others.

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Promega Corporation · 2800 Woods Hollow Road · Madison, WI 53711-5399 USA · Toll Free in USA 800-356-9526 · 608-274-4330 · Fax 608-277-2516
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