TECHNICAL MANUAL

ONE-Glo™ Luciferase Assay System

Instructions for Use of Products
E6110, E6120 and E6130

Revised 1/16
TM292

ONE-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

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1. Description

High- and ultrahigh-throughput quantitation of luciferase expression in mammalian cells requires sensitive reagents. The ONE-Glo™ Luciferase Assay System[a,b] employs new assay chemistry to provide a robust, homogeneous assay. A new luciferase substrate has enabled Promega to create a novel reagent that is more robust and stable when reconstituted, and less aromatic than standard reagents created with luciferin (see Section 5.A).

The ONE-Glo™ Luciferase Assay Buffer and ONE-Glo™ Luciferase Assay Substrate, provided with this system, are combined to form the ONE-Glo™ Reagent (see Section 3.B).

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
ONE-Glo™ Luciferase Assay System	**10ml**	**E6110**

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

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

PRODUCT	SIZE	CAT.#
ONE-Glo™ Luciferase Assay System	**100ml**	**E6120**

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

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

PRODUCT	SIZE	CAT.#
ONE-Glo™ Luciferase Assay System	**1L**	**E6130**

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

- 1L ONE-Glo™ Luciferase Assay Buffer
- 1 vial ONE-Glo™ Luciferase Assay Substrate (lyophilized)

Storage Conditions: Store the ONE-Glo™ Luciferase Assay System components at –20°C. The substrate can also be stored at room temperature for up to 3 weeks or at 4°C for up to 3 months with only a 12% decrease in functionality. Store the ONE-Glo™ Buffer at 4°C. The buffer can also be stored at room temperature for up to 3 months with approximately a 10% change in reagent functionality. For convenience the buffer label contains a space on which to record the date the buffer is transferred to room temperature. Store the buffer at room temperature to prevent the need for temperature equilibration when the reagent is reconstituted.

3. Performing the ONE-Glo™ Luciferase Assay

3.A. General Considerations

The ONE-Glo™ Luciferase Assay System is designed for use with the following culture media containing 0–10% serum: RPMI 1640, DMEM, MEMα, Ham's F12, AmnioMax, CD CHO, CD 293. The signal half-life under these conditions will generally exceed 45 minutes at 22°C and is independent of the enzyme concentration. Other medium/serum combinations can also be used but experimental verification of assay performance is recommended in these cases (see Section 5.B). The luminescence signal can also be affected by changes in temperature or by the presence of organic solvents (see Section 5.B).

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

Because the luminescence signal is affected by assay conditions, results should be compared only between samples measured using the same media/serum combinations. For analysis of multiple plates, the greatest accuracy can be obtained by incorporation of 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 for the correction of small variations in luminescence that can occur over time or due to other variables such as temperature.

A short amount of time is required for cell lysis; therefore ONE-Glo™ Luciferase Assay Reagent should be added to plates at least 3 minutes prior to quantifying luminescence. For maximal light intensity, samples should be measured within 30 minutes of reagent addition. ONE-Glo™ Reagent is recommended for measuring luminescence in experiments where the measurement step takes less than 2.5 to 3 hours. In the media tested, the luminescence generated by the ONE-Glo™ Reagent was brighter than that generated by the Steady-Glo® Reagent over this period (see Section 5.A).

ONE-Glo™ Reagent is not designed for use with the automated reagent injectors integrated into some luminometers.

To achieve linear assay performance at low light levels, the background luminescence must be subtracted from all readings. No background is produced by the ONE-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 ONE-Glo™ Reagent after reconstitution: 18% loss of luminescence over 24 hours at room temperature and 12% loss over 5 days at 4°C. No change in functionality was measured after 9 weeks at –20°C. The reagent can be subjected to up to 10 freeze-thaw cycles with no effect on functionality.

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3.B. Reagent Preparation

For Cat.# E6110 and E6120, transfer the contents of one bottle of ONE-Glo™ Buffer to one bottle of ONE-Glo™ Substrate. Mix by inversion until the substrate is thoroughly dissolved. This may require multiple inversions.

For Cat.# E6130, transfer some of the ONE-Glo™ Buffer to the bottle of ONE-Glo™ Substrate and invert to dissolve the substrate. Be careful not to overfill the substrate bottle, which only holds approximately one quarter of the 1L of buffer. When the substrate has been dissolved, pour the mixture back into the buffer bottle and mix by inverting.

Notes:

1. Since luciferase activity is temperature dependent, the temperature of the ONE-Glo™ Reagent should be kept constant while quantitating luminescence. This is most easily accomplished by using reagent that is equilibrated to room temperature. Equilibration of the reagent prior to use is unnecessary when the buffer is stored at room temperature.
2. If the reagent is stored at 4°C or frozen after reconstitution, it must be warmed or thawed at temperatures below 25°C to ensure optimal performance. The most convenient and effective method for thawing or temperature equilibrating cold reagent is by placing the reagent in a water bath at room temperature. Mix well after thawing.
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 measurement in the luminometer being used.
2. Add a volume of reagent equal to that of the culture medium in each well. Mix for optimal consistency. For 96-well plates, typically 100µl of reagent is added to the 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 3 minutes to allow complete cell lysis and measure in a luminometer (consult the instrument manual).

4. Related Products

Luciferase Assay Systems

Product Name	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
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

Miscellaneous Luciferase Products

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

4. Related Products (continued)

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
pGL4.14[*luc2*/Hygro] Vector	20µg	E6691
pGL4.15[*luc2P*/Hygro] Vector	20µg	E6701
pGL4.16[*luc2CP*/Hygro] Vector	20µg	E6711
pGL4.17[*luc2*/Neo] Vector	20µg	E6721
pGL4.18[*luc2P*/Neo] Vector	20µg	E6731
pGL4.19[*luc2CP*/Neo] Vector	20µg	E6741
pGL4.20[*luc2*/Puro] Vector	20µg	E6751
pGL4.21[*luc2P*/Puro] Vector	20µg	E6761
pGL4.22[*luc2CP*/Puro] Vector	20µg	E6771
pGL4.23[*luc2*/minP] Vector	20µg	E8411
pGL4.24[*luc2P*/minP] Vector	20µg	E8421
pGL4.25[*luc2CP*/minP] Vector	20µg	E8431
pGL4.26[*luc2*/minP/Hygro] Vector	20µg	E8441
pGL4.27[*luc2P*/minP/Hygro] Vector	20µg	E8451
pGL4.28[*luc2CP*/minP/Hygro] Vector	20µg	E8461
pGL4.29[*luc2P*/CRE/Hygro] Vector	20µg	E8471
pGL4.30[*luc2P*/NFAT-RE/Hygro] Vector	20µg	E8481
pGL4.31[*luc2P*/*Gal4*UAS/Hygro] Vector	20µg	C9351

GloResponse™ Luciferase Reporter Cell Lines

Product	Size	Cat.#
GloResponse™ CRE-luc2P HEK293 Cell Line	2 vials	E8500
GloResponse™ NFAT-RE-luc2P HEK293 Cell Line	2 vials	E8510

5. Appendix

5.A. Overview of the ONE-Glo™ Luciferase Assay

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 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, can also 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 magnesium, 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 (including fireflies). The enzyme uses ATP as a co-factor 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 (see Section 4).

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

Development of the Assay

Promega has created a new chemical approach to the firefly luciferase reaction, incorporating a new luciferin analog as the substrate (Figure 2). The new chemistry generates bright luminescence that is stable enough for easy measurement (Figure 3) and provides additional advantages to the ONE-Glo™ Luciferase Reagent: i) the reagent has much less odor from thiol than conventional luciferase reagents; ii) the reconstituted reagent and the reagent substrates are functionally much more stable than similar luciferin-containing reagents; and iii) the reagent is more tolerant of non-substrate components in the luciferase reaction, for example it is tolerant of phenol red.

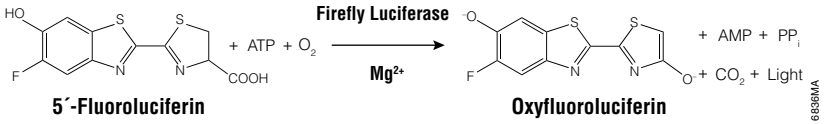

Figure 2. The luciferase reaction utilizing 5´-fluoroluciferin.

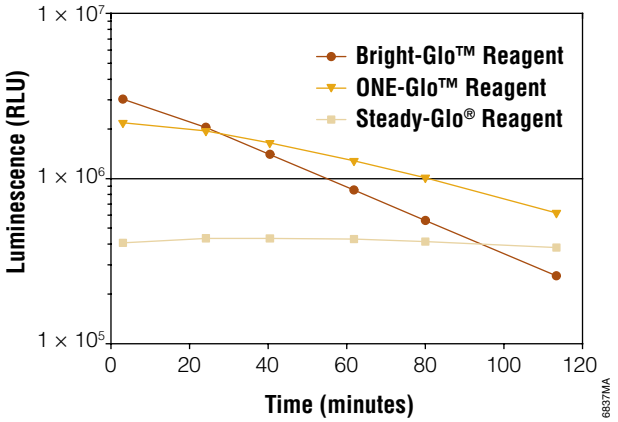

Figure 3. ONE-Glo™ Reagent generates bright and stable luminescence that is measurable for hours. Samples in 96-well plates consisted of 50µl of purified firefly luciferase (14.9ng/ml in DMEM with 0.1% Prionex® as carrier) combined with 50µl of the respective reagent. Luminescence was measured (1.0 second integration/well) at 3 minutes and periodically over almost 2 hours. All coefficients of variation were < 3%; n = 3.

Decreased Thiol Odor

Because it contains fluoroluciferin, the ONE-Glo™ Reagent does not require the high concentrations of DTT that cause conventional luciferase reagents to have a strong odor. The ONE-Glo™ Reagent contains almost 100-fold less odor-causing thiol than the conventional homogeneous reagents.

Increased Stability of Components and Reconstituted Reagent

The ONE-Glo™ Reagent is more convenient to use, store and reuse than other luciferase reagents. The presence of fluoroluciferin results in more physical stability of the ONE-Glo™ Reagent than that seen with conventional bright-type luciferin-containing reagents. In order to be useful as a substrate for luciferase, the luciferin substrate must be deprotonated. The fluorine acts as an electron acceptor in the 5´-fluoroluciferin, keeping the fluoroluciferin predominantly deprotonated at neutral and slightly acidic pH. The fluoroluciferin, therefore, is a more active substrate than luciferin at these pHs (Figure 4).

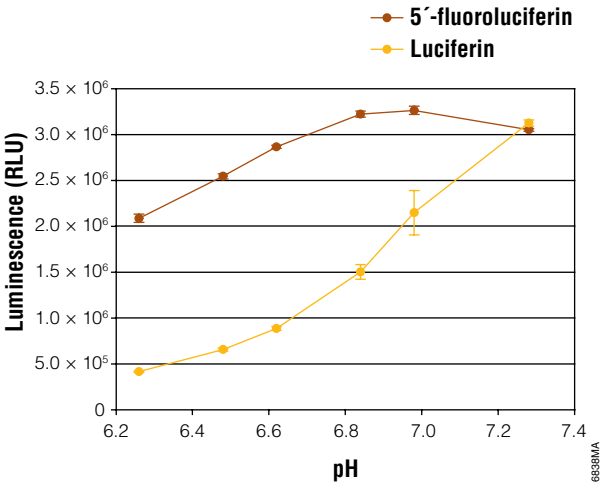

Figure 4. Luciferase reactions containing 5´-fluoroluciferin generate more light than those containing luciferin at low pH. The pH of the ONE-Glo™ Reagent formulation lacking 5´-fluoroluciferin was adjusted to between 6.26 and 7.28. 5´-fluoroluciferin or luciferin was then added to the reagents. Reactions were initiated by adding an equal volume of DMEM containing 0.1% Prionex® and 14.9ng/ml luciferase. Measurements were made 3 minutes after reaction initiation; n = 3.

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

The main route of luciferase reagent inactivation is thought to be oxidation, and the acidic pH of the ONE-Glo™ Reagent permits the reconstituted reagent to remain functionally stable longer. Luciferin-based reagents that generate high luminescence, such as the bright-type chemistries, decrease in functionality very quickly, commonly by 10% every 4–5 hours at 22°C. ONE-Glo™ Reagent functionality decreases at one-third of that rate or less, by approximately 10% every 12–20 hours at 22°C (Figure 5). Similar benefits are seen at 4°C, where the ONE-Glo™ Reagent decreases functionally by about 10% in 5 days.

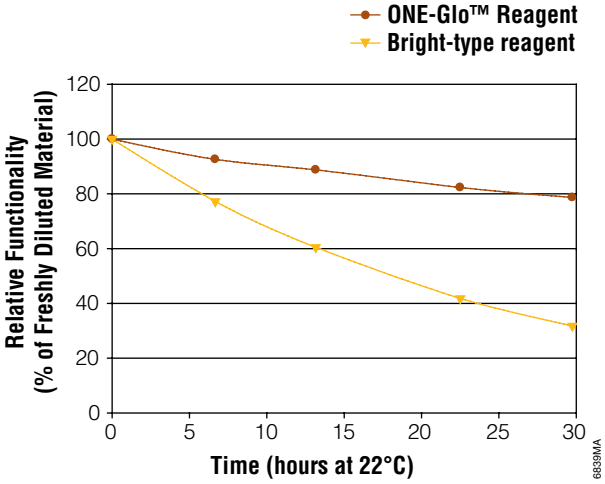

Figure 5. Reconstituted ONE-Glo™ Reagent can be used much longer than other commercially available bright-type reagents. Reconstituted reagents were stored at 22°C and frozen at –70°C at defined times. Upon thawing, the reagents were combined with an equal volume of DMEM containing 14.9ng/ml QuantiLum® Luciferase and 0.1% Prionex®. The relative functionality was calculated as the luminescence signal intensity for each sample, measured 3 minutes after enzyme addition, relative to the signal intensity of the sample that was placed at –70°C without 22°C incubation; n = 3.

Lower pH also helps increase the stability of the lyophilized substrates. The ONE-Glo™ Luciferase Substrate is far more stable than the conventional luciferase reagent substrates. The ONE-Glo™ Substrate can remain at room temperature for 3 weeks with only an 11% decrease in functionality, and it can remain at 4°C for months before a decrease in functionality can be measured.

Increased Tolerance to Non-Substrate Reaction Components

The ONE-Glo™ Reagent also is more tolerant of non-substrate components in the reaction environment. When homogeneous luciferase reagents are used, the media, serum (if present) and any experimental chemical compounds are not removed from cells prior to adding the luciferase reagent. However, the best representation of the amount of luciferase present occurs when data is minimally affected by these non-substrate components present in the reaction mixture. The ONE-Glo™ Reagent was designed to reduce the effects of non-substrate reaction components, specifically experimental chemical compounds. Known luciferase inhibitors like resveratrol (6) have been tested in reactions containing ONE-Glo™ or other bright-type reagents. With ONE-Glo™ Reagent the luciferase reaction retains 86% of its activity in the presence of 10µM resveratrol, while with other bright-type reagents the reaction retains only 21% of its activity (Figure 6).

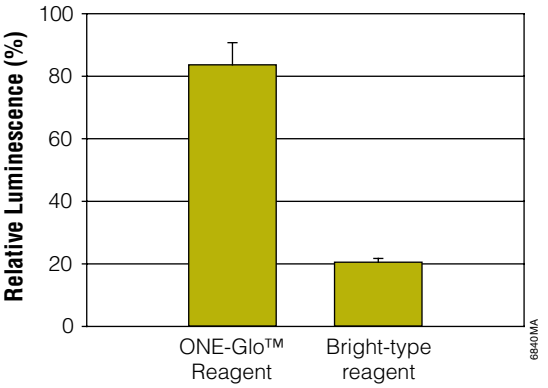

Figure 6. ONE-Glo™ Reagent protects the luciferase reaction from inhibition by 10µM resveratrol. Luciferase reactions generated by ONE-Glo™ or another commercially-available bright-type reagent were initiated in the presence or absence of 10µM resveratrol. Luminescence was initiated and measured as described in Figure 4. The relative luminescence equals the luminescence of reactions containing the resveratrol divided by the luminescence of reactions without resveratrol; n = 3.

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

This increase in tolerance also makes the ONE-Glo™ Reagent more convenient to use than other luciferase reagents. Reactions containing ONE-Glo™ Reagent are minimally affected by the non-substrate reaction components, such as phenol red (Figure 7). The effects of typical media components and solvents are shown in Section 5.B. The effects of temperature on the luciferase reaction also are discussed in this section.

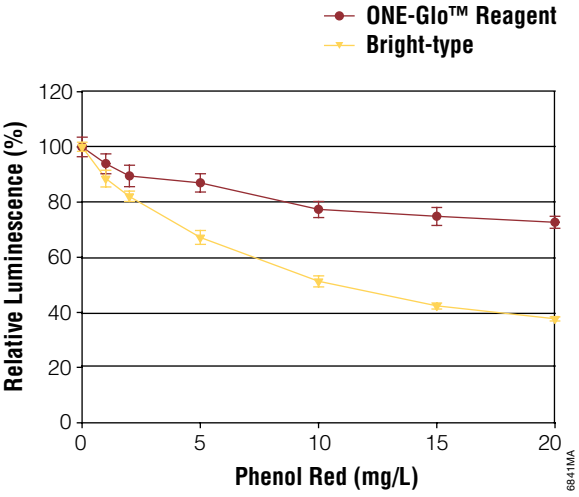

Figure 7. ONE-Glo™ Reagent is more tolerant of phenol red than other luciferin-based reagents. Luciferase reactions composed of 14.9ng/ml luciferase in phenol red-free DMEM/F12 medium (with 0.1% Prionex®) plus ONE-Glo™ Reagent or another commercially-available bright-type reagent, were initiated in the presence of varied amounts of phenol red. Relative luminescence is the luminescence of reactions containing the phenol red divided by the luminescence from reactions without phenol red; n = 3.

5.B. Effects of Typical Reaction Components

The data provided in this section are intended to provide a general overview of the assay characteristics under a wide range of experimental conditions. Please note that the ONE-Glo™ Reagent is chemically different than other luciferase assays. Therefore, the data presented here may not be applicable to other luciferase assay systems.

Most of the data presented in this section was generated using purified luciferase diluted into culture medium. This was done to illustrate performance characteristics of the reagent while avoiding experimental complexities common to cell culture. As seen in Figure 8, the purified luciferase reactions showed little or no difference from enzyme expressed in transfected cells.

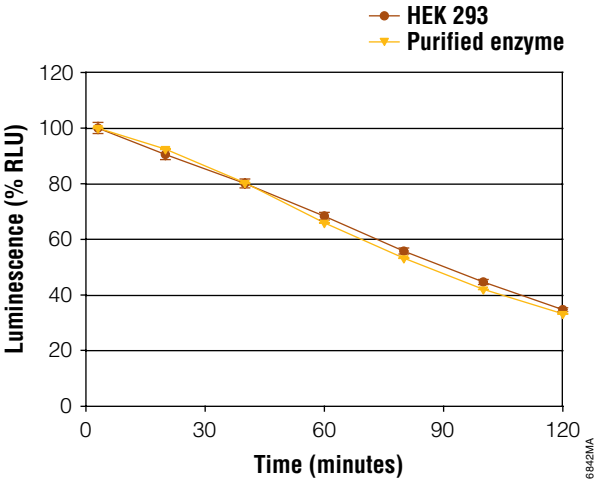

Figure 8. Similar luminescence kinetics are generated from purified enzyme and from luciferase expressed from stably transfected mammalian cells. Samples in 96-well plates consisted either of 100µl of purified enzyme (14.9ng/ml plus 0.1% Prionex®) or HEK 293 cells (10,000 cells per well) that have been stably transfected with the luciferase reporter gene under control of the NFAT-response element. As these data show, signal kinetics are very similar for purified enzyme and luciferase expressed by cells bathed in the same medium (DMEM + 10% FBS).

5.B. Effects of Typical Reaction Components (continued)

Culture Medium

Like other homogeneous luciferase assays, half of the reaction volume for ONE-Glo™ Reactions is mammalian tissue culture medium. The ONE-Glo™ Reagent is designed to work well with a variety of common media. However, differences between media can affect the intensity and duration of the luminescent signal (Figure 9). Differences in media or between different manufacturers or lots of the same media make incorporating controls in each batch of plates advisable.

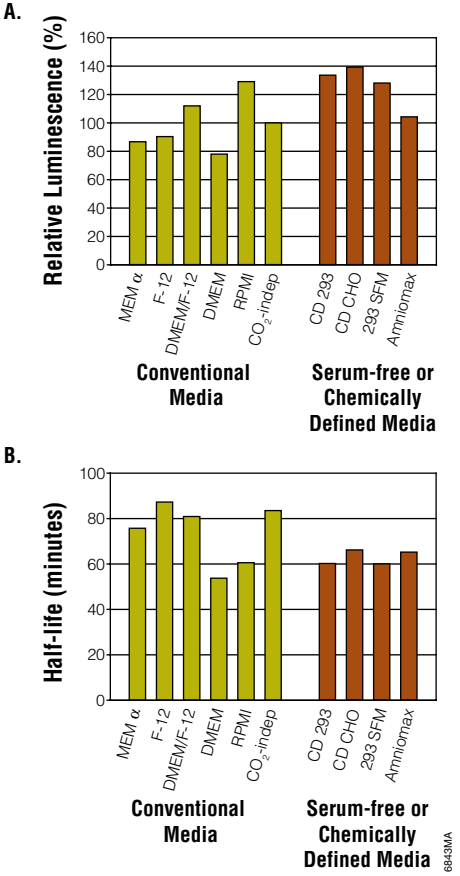

Figure 9. Relative intensity and signal stability of firefly luciferase in assorted common media. Purified firefly luciferase (14.9ng/ml in medium with 0.1% Prionex®) was added to reactions. Luminescence was measured 3 minutes after enzyme addition and periodically for 2 hours. **Panel A.** Luminescence (at 3 minutes) is shown relative to that measured from CO_2-independent medium. **Panel B.** Signal stability in different media expressed as half-life of the log-linear portion of the curve (after approximately 30 minutes, see Figure 3).

Serum

ONE-Glo™ Luciferase Assay Reagent is compatible with serum in media. The reagent was designed for use with 0–10% serum in media, and the luminescence generated is minimally affected at these concentrations (Figure 10).

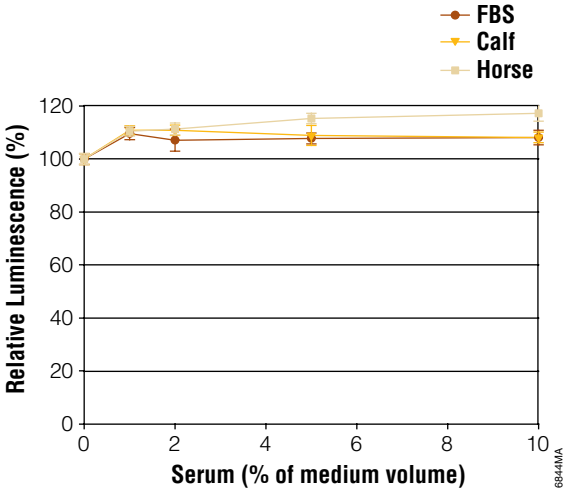

Figure 10. Reactions with ONE-Glo™ Reagent are generally unaffected by serum. Luciferase reactions composed of 14.9ng/ml luciferase in DMEM medium with 0.1% Prionex® and ONE-Glo™ Reagent were initiated in the presence of varied amounts of fetal bovine serum (FBS), calf serum or horse serum. The retained activity (relative luminescence) is the luminescence of reactions containing the serum divided by luminescence from reactions without serum; n = 3.

5.B. Effects of Typical Reaction Components (continued)

Organic Solvents

Organic solvents are typically present in luciferase assays as solvents for experimental compounds. Typical solvents like DMSO, methanol and ethanol have little effect on the luminescence generated by reactions with ONE-Glo™ Reagent (Figure 11).

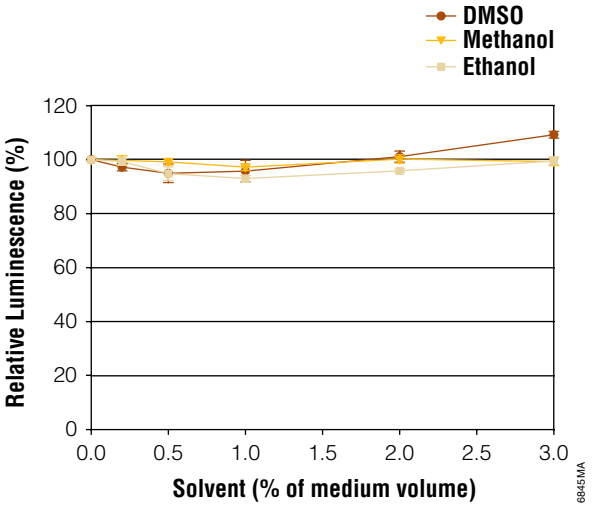

Figure 11. Reactions with ONE-Glo™ Reagent are generally unaffected by solvents. Luciferase reactions composed of 14.9ng/ml luciferase in DMEM medium with 0.1% Prionex® and ONE-Glo™ Reagent were initiated in the presence of varied amounts of solvent. The relative luminescence is the luminescence of reactions containing the solvent divided by the luminescence of reactions without solvent; n = 3.

Temperature

Luciferase activity is temperature dependent, thus temperature is an important factor in experimental precision (Figure 12). Good precision can be achieved most easily by performing all experiments at room temperature, as this is near the temperature optimum for firefly luciferase activity. The assay reagent should be at room temperature before beginning measurements.

As mentioned previously, all the ONE-Glo™ components may be stored at room temperature for about 3 weeks with minimal effect on functional stability. Furthermore, the ONE-Glo™ Buffer may be stored at room temperature for approximately 3 months with little effect on functionality. Storage at room temperature avoids the need to equilibrate the reconstituted reagent to room temperature before experimentation. If the components or reconstituted reagent has been stored chilled, warming in a room temperature water bath speeds equilibration. Do not use a water bath at temperatures above 25°C.

Regarding the effects of temperature on luminescence signal intensity, higher temperatures cause higher signal intensity but lower signal stability, while lower temperatures cause lower signal intensity but higher signal stability (Figure 12). Reaction temperature can be affected by chilled reagent, by culture plates that are too warm, by excess heat within luminometers when making many reads, and other factors. Control samples on each plate can be used to normalize these effects between plates in an experiment.

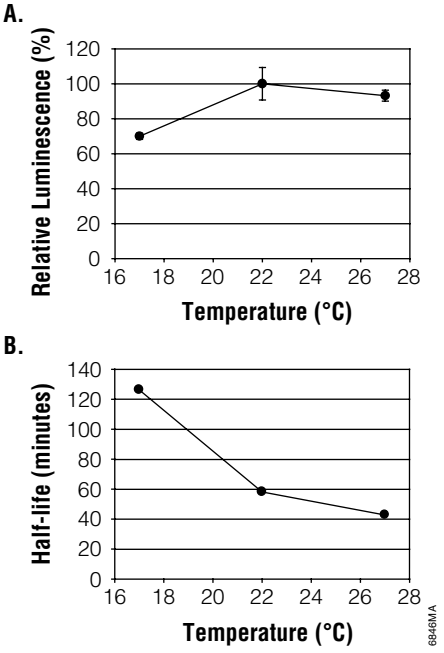

Figure 12. Effect of temperature on luminescence. Reactions containing purified firefly luciferase (14.9ng/ml in DMEM plus 0.1% Prionex) and ONE-Glo™ Reagent were equilibrated to temperatures of 17°, 22° or 27°C before reactions were initiated. Three minutes after the reactions were begun and periodically over the next 2 hours, luminescence was measured. **Panel A.** Luminescence (at 3 minutes) is shown relative to that measured at 22°C. **Panel B.** Signal half-life, calculated from data generated after 30 minutes, is shown; n = 3 and the coefficients of variation are < 5%.

5.C. References

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6. Summary of Changes

The following change was made to the 1/16 revision of this document:

1. Patent and disclaimer statements were updated.

[a]Patent Pending.

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