TECHNICAL MANUAL

ONE-Glo™ EX Luciferase Assay System

Instructions for Use of Products
E8110, E8120, E8130 and E8150

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TM432

ONE-Glo™ EX 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

High- or ultrahigh-throughput quantitation of luciferase expression in mammalian cells is commonly performed by measuring luminescence from 96-, 384- or 1,536-well plates. The ONE-Glo™ EX Luciferase Assay System[a,b] provides both the high sensitivity and long-lived luminescence required to batch process multiple plates in these assay formats. The ONE-Glo™ EX Assay retains many of the beneficial aspects of the ONE-Glo™ Assay, using 5´-fluoroluciferin as substrate with an add-mix-read, or homogeneous, protocol. Extending the properties of ONE-Glo™ Reagent, ONE-Glo™ EX Reagent employs a new assay chemistry to increase both the stability of the luminescence signal and greatly increase the stability of the reconstituted reagent. The approximately 2 hour signal half-life provides greater flexibility in assay design. A reconstituted reagent that can be stored at room temperature for longer periods means less variability in reagent performance during long experiments or screens and less sample waste. ONE-Glo™ EX Reagent is the firefly luciferase detection reagent used in the Nano-Glo® Dual-Luciferase® Reporter (NanoDLR™) Assay System, allowing the same reagent to be used for single- or dual-luciferase assays.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
ONE-Glo™ EX Luciferase Assay System	**10ml**	**E8110**

Each system contains sufficient components to prepare 10ml of reagent. Includes:

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

PRODUCT	SIZE	CAT.#
ONE-Glo™ EX Luciferase Assay System	**100ml**	**E8120**

Each system contains sufficient components to prepare 100ml of reagent. Includes:

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

PRODUCT	SIZE	CAT.#
ONE-Glo™ EX Luciferase Assay System	**10 × 10ml**	**E8130**

Each system contains sufficient components to prepare 100ml of reagent. Includes:

- 10 × 10ml ONE-Glo™ EX Luciferase Assay Buffer
- 10 vials ONE-Glo™ EX Luciferase Assay Substrate (lyophilized)

PRODUCT	SIZE	CAT.#
ONE-Glo™ EX Luciferase Assay System	**10 × 100ml**	**E8150**

Each system contains sufficient components to prepare 1,000ml of reagent. Includes:

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

Storage Conditions: Store the ONE-Glo™ EX Luciferase Assay components at –10°C to –30°C. The ONE-Glo™ EX Luciferase Assay Buffer may be stored at 4°C for 1 year or at room temperature for 6 months.

Reconstituted ONE-Glo™ EX Reagent can be stored at 4°C or –20°C for later use, protected from light. Warm or thaw reagent at temperatures below 25°C to ensure optimal performance (e.g., place the reagent in a water bath at room temperature). Mix by inversion after thawing. ONE-Glo™ EX Reagent will lose 10% activity in approximately 18 hours and 50% activity in approximately 6 days at 22°C. At 4°C, the ONE-Glo™ EX Reagent will lose 10% activity in approximately 3.5 days and 50% activity in approximately 1 month.

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3. Performing the ONE-Glo™ EX Luciferase Assay

3.A. General Considerations

The ONE-Glo™ EX Luciferase Assay System has been designed to be used with many media types and has been verified for use with the following culture media containing 0–10% serum: DMEM, RPMI 1640, McCoy's 5A and F-12. While the reagent should give a signal half-life of approximately 2 hours at 22°C in many media types, different combinations of media and serum may affect the luminescence or signal decay rate (see Section 5.B). The luminescence can also be affected by the presence of phenol red, organic solvents and changes in temperature (Section 5.B).

Because luminescent signals are affected by assay conditions, raw results should be compared only between samples measured at the same time and using the same medium and serum combination. For analysis of multiple plates, the greatest accuracy can be obtained by incorporating a common control sample in each plate. This corrects the small variations in luminescence that can occur over time or due to other variables such as temperature.

To achieve linear assay performance at low light levels, subtract the background luminescence from all readings. Background luminescence is a characteristic of luminometer performance as the ONE-Glo™ EX Reagent and mammalian cells lacking the luciferase gene produce no background. Some instruments also require verification of linear response at high light levels (consult the instrument manual).

3.B. Reagent Preparation

Transfer the contents of one bottle of ONE-Glo™ EX Buffer to one bottle of ONE-Glo™ EX Substrate. Replace the stopper and mix by inversion until the substrate is thoroughly dissolved. This should take less than 10 seconds.

Notes:

1. Due to the temperature dependency of luciferase activity, the temperature of the samples and the reagent should be kept constant while measuring luminescence. Ensure that the reagent is equilibrated to room temperature before use (e.g., placing the buffer at room temperature at least a day before experiments). Equilibrate cultured cells to room temperature before adding the reagent.
2. If the reconstituted ONE-Glo™ EX Reagent is stored at 4°C or frozen, warm or thaw the reagent at temperatures below 25°C to ensure optimal performance (e.g., place the reagent in a water bath at room temperature). Mix by inversion after thawing.
3. Once reconstituted, the ONE-Glo™ EX Reagent will lose 10% activity in approximately 18 hours and 50% activity in approximately 6 days at 22°C. Unused reconstituted reagent can be stored at 4°C or –20°C for later use, protected from light. At 4°C, the ONE-Glo™ EX Reagent will lose 10% activity in approximately 3.5 days and 50% activity in approximately 1 month.

3.C. Assay Procedure

1. Remove plates from the incubator and equilibrate to room temperature. Use an opaque white tissue-culture plate to minimize cross-talk between wells and absorption of emitted light.

   ! Ensure that the plates are compatible with the type of luminometer being used.

2. Add a volume of ONE-Glo™ EX Reagent that is equal to the volume of culture medium in each well, and incubate samples for at least 3 minutes to lyse cells and equilibrate samples. For optimal results, mix samples by placing the plate on an orbital shaker at 300–600rpm for 1–3 minutes. For 96-well plates, typically 80–100µl volumes are used. For 384-well plates, 20–30µl volumes are typically used.

3. Measure luminescence in a luminometer or instrument capable of measuring luminescence.

   Notes:

   1. Consult the instrument manual for instructions. We recommend an integration time of 0.5–1 second for 96-well plates when using GloMax® instruments.
   2. For optimal results, measure luminescence within 2 hours of adding ONE-Glo™ EX Reagent. The luminescence intensity has a signal half-life of approximately 2 hours.

4. Firefly Luciferase Vectors for Use with the ONE-Glo™ EX Luciferase Assay System

To select a firefly luciferase reporter vector suitable for use with the ONE-Glo™ EX Luciferase Assay System, visit: **www.promega.com/luciferase-vectors**

5. Appendix

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

Reporter genes are routinely used to study a wide range of physiological events. Examples include the study of regulated gene expression and signal transduction, where reporter protein expression is used as a surrogate to monitor changes in gene transcription. Luciferase is a popular choice as a reporter for these applications because functional enzyme is created immediately upon translation, and the assay is rapid and easy to perform. Furthermore, the sensitivity and linearity of luciferase detection are unmatched when compared to alternative reporter proteins. For these reasons, luciferase is widely used in the biotechnology and pharmaceutical industries, including the automated platforms for high-throughput screening used in drug discovery (1).

Firefly luciferase is a 61kDa monomer that catalyzes the mono-oxygenation of beetle luciferin. The enzyme uses ATP and molecular oxygen as cosubstrates. The ONE-Glo™ EX Reagent uses a new assay chemistry that extends the properties of ONE-Glo™ Reagent to generate a bright luminescent signal with improved signal and reagent stability. The use of the luciferin analog, 5´-fluoroluciferin (Figure 1), in the ONE-Glo™ and ONE-Glo™ EX Assay Systems allows the assays to be performed at a lower pH. The new ONE-Glo™ EX assay chemistry provides several advantages over similar luciferase reagents including: 1) increased reconstituted reagent stability; 2) improved ONE-Glo™ EX Reagent signal half-life, 1.5-fold longer than that of ONE-Glo™ Reagent; 3) reduced quenching of luminescence by phenol red in cell culture medium; 4) eliminated odor-causing thiol compounds, such as DTT, in the reagent.

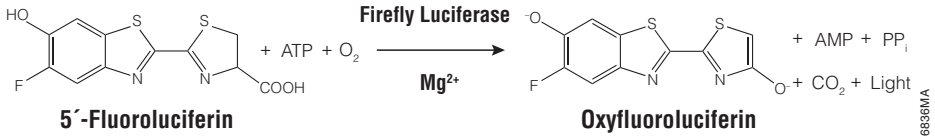

Figure 1. The bioluminescent reaction catalyzed by firefly luciferase in the ONE-Glo™ EX Assay.

To facilitate batch processing of plates and decrease variability within an experiment, the ONE-Glo™ EX reagent generates a bright, stable luminescent signal that decays at a steady rate with a half-life of approximately two hours in many media types (Figures 2 and 4). Compared to ONE-Glo™ Reagent, ONE-Glo™ EX Reagent produces a slightly dimmer signal (approximately 2/3 that of ONE-Glo™ Reagent), but with a compensating increase in the signal half-life (~115 versus ~75 minutes on average).

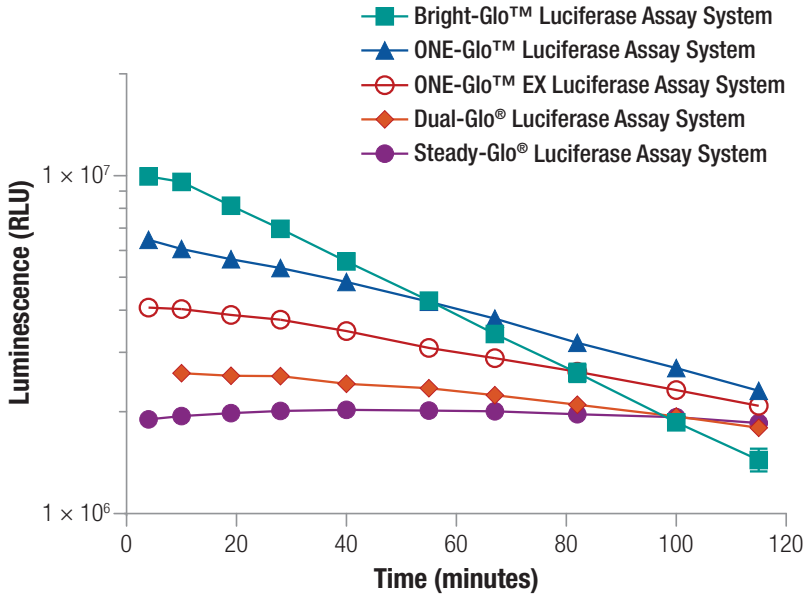

Figure 2. ONE-Glo™ EX Reagent provides bright and stable luminescence that can be measured for hours. One hundred microliters of purified firefly luciferase (13.8ng/ml in DMEM with 0.1% Prionex® as carrier) was combined in a 96-well plate with 100µl of Bright-Glo™, ONE-Glo™, ONE-Glo™ EX, Dual-Glo® Luciferase or Steady-Glo® Reagents. Luminescence was measured periodically over 2 hours, n = 8.

ONE-Glo™ EX Reagent was designed to provide extended stability once reconstituted compared to similar reagents, enabling more constant luminescence over long experiments. This reagent stability makes it more convenient to use, store and reuse than other luciferase reagents, reducing waste. Once reconstituted, ONE-Glo™ EX Reagent will lose 10% of its activity at 22°C after about 18 hours and lose 50% of its activity after about 6 days (Figure 3). Unused reagent can be stored at –20°C, but avoid multiple freeze-thaw cycles. The reagent can also be stored at 4°C, at which temperature it will lose 10% activity after about 3.5 days and 50% activity after about a month. After even prolonged incubation at room temperature (>3 weeks), the reagent may still retain about 25% of its original activity.

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

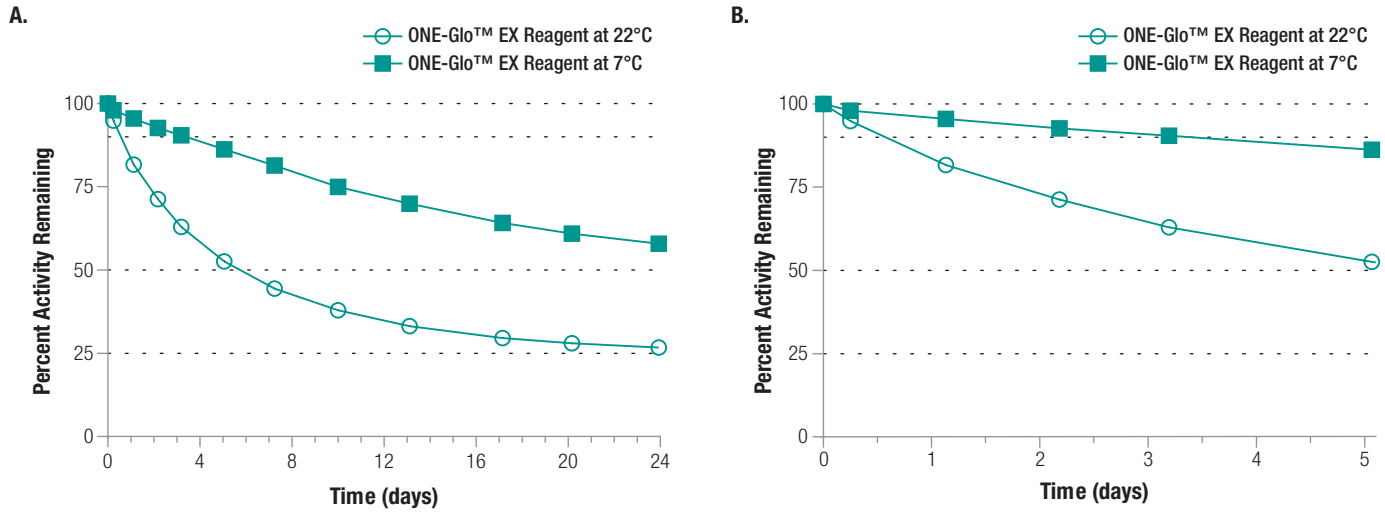

Figure 3. ONE-Glo™ EX Reagent displays extended stability once reconstituted. Reconstituted reagent was stored at 22°C or 7°C and frozen at –70°C at defined times. Upon thawing and equilibrating to room temperature, the ONE-Glo™ EX Reagent samples were combined with an equal volume of 13.8ng/ml purified firefly luciferase in DMEM supplemented with 10% fetal bovine serum (FBS). 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 with no incubation at 22°C. The activity data is shown over 24 days (**Panel A**) and over just the first 5 days (**Panel B**); n=4.

5.B. Effects of Typical Reaction Conditions

Culture Medium

Like other add-mix-measure luciferase assays, half of the reaction volume for ONE-Glo™ EX reactions is mammalian tissue culture medium. The ONE-Glo™ EX 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 4). For instance, the phenol red in some media may decrease signal intensity. Differences in media or between different manufacturers or lots of the same medium make it important to incorporate controls in each batch of plates.

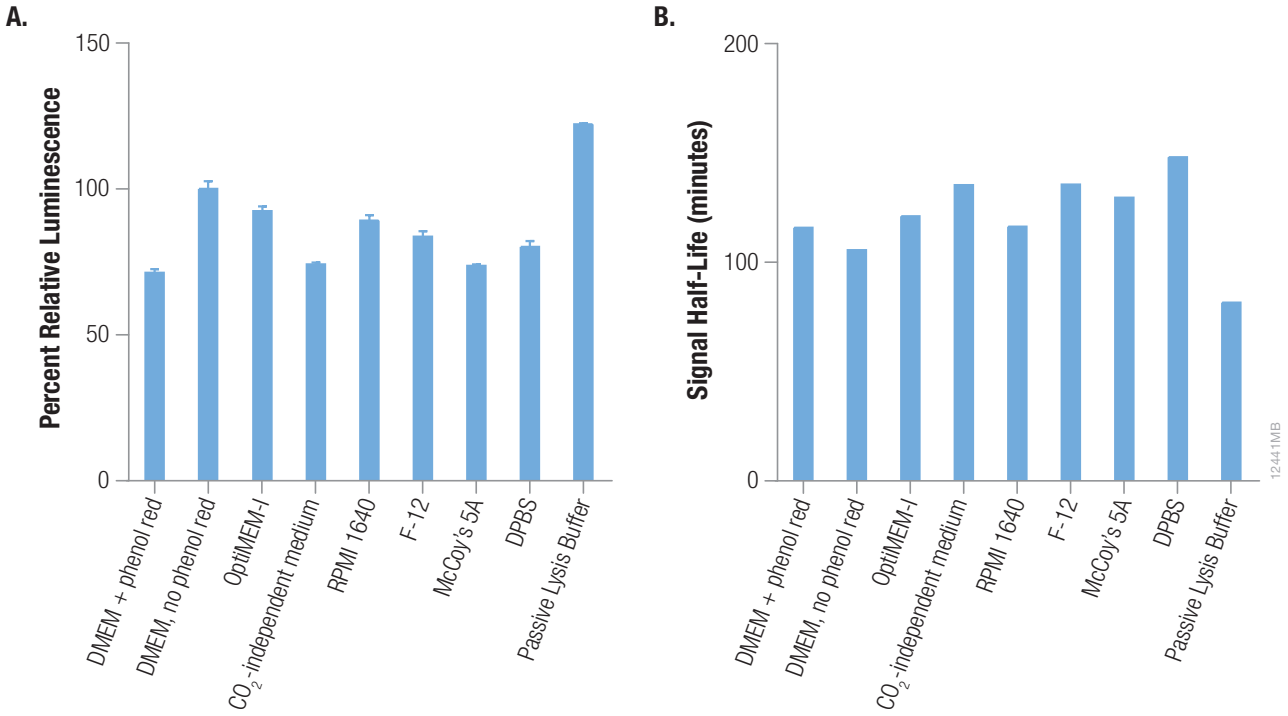

Figure 4. Relative luminescence intensity and signal stability of firefly luciferase in common media types. HEK293 cells were transiently transfected with firefly luciferase expression constructs. Cells were harvested, split equally among tubes, centrifuged and resuspended to 3.4×10^5 cells/ml in various media types supplemented with 10% fetal bovine serum (FBS) with the exception of Passive Lysis Buffer, which contained no serum and was allowed to incubate with the cells for 15 minutes. Eighty microliters of cells were added to the wells of a 96-well plate before dispensing 80µl of ONE-Glo™ EX Reagent. Luminescence was measured periodically over 2.5 hours. **Panel A.** The firefly luminescence at 3 minutes is shown relative to that measured from DMEM without phenol red; n = 4. **Panel B.** The signal stability in different media is expressed as the half-life of the signal decay over the 2.5 hours.

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

Serum

The ONE-Glo™ EX Reagent has been designed for use with 0–10% serum, and the luminescent signals generated are minimally affected by the presence of serum (Figure 5).

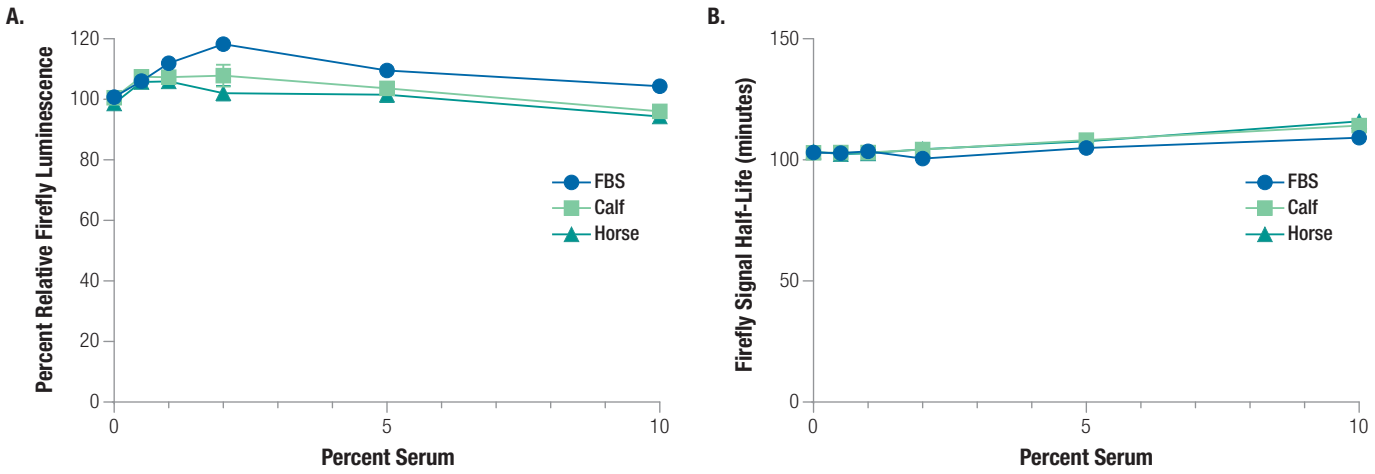

Figure 5. Effects of serum on luminescence intensity and signal stability. Purified firefly luciferase (6.88ng/ml) was diluted into DMEM plus 0.1% Prionex® with varying concentrations of fetal bovine serum (FBS), calf serum or horse serum. Enzyme solutions (80µl per well) were added to 96-well plates, then 80µl of ONE-Glo™ EX Reagent was dispensed. Luminescence was measured periodically over 2 hours to calculate the signal stability (half-life); n = 4. **Panel A.** Firefly luminescence relative to no serum. **Panel B.** Luminescent signal half-life.

Organic Solvents

Organic solvents may be present in reporter gene assays because they are used to solubilize screening compounds. DMSO, ethanol and methanol in concentrations up to 3% have little effect on luciferase luminescence or signal kinetics (Figure 6).

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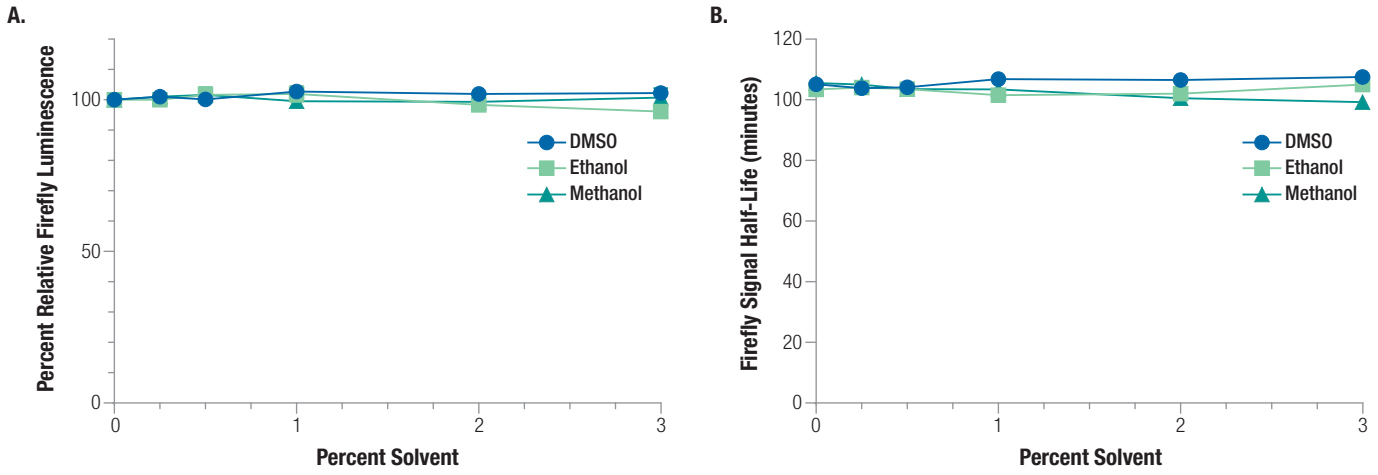

Figure 6. The effect of organic solvents on luminescence intensity and signal stability. Purified firefly luciferase (6.88ng/ml) was diluted into DMEM plus 0.1% Prionex® with varying concentrations of dimethyl sulfoxide (DMSO), ethanol or methanol. Enzyme solutions (80µl per well) were added to 96-well plates, and then 80µl of ONE-Glo™ EX Reagent was dispensed. Luminescence was measured periodically over 2 hours to calculate the signal stability (half-life); n = 4. **Panel A.** Firefly luminescence relative to no solvent. **Panel B.** The signal half-life.

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

Phenol Red

Phenol red is a pH indicator commonly used in cell culture media. Many commercial medium formulations contain 5–15mg/l phenol red, causing the characteristic red color. Because phenol red can absorb light, it may reduce assay sensitivity. However, the lower pH of the ONE-Glo™ EX Reagent makes it less sensitive to phenol red compared to other luciferase reagents (Figure 7). For most applications, the presence of phenol red will not significantly affect the the ONE-Glo™ EX Assay. However, to maximize the luminescent signal, use as little phenol red as possible in culture medium.

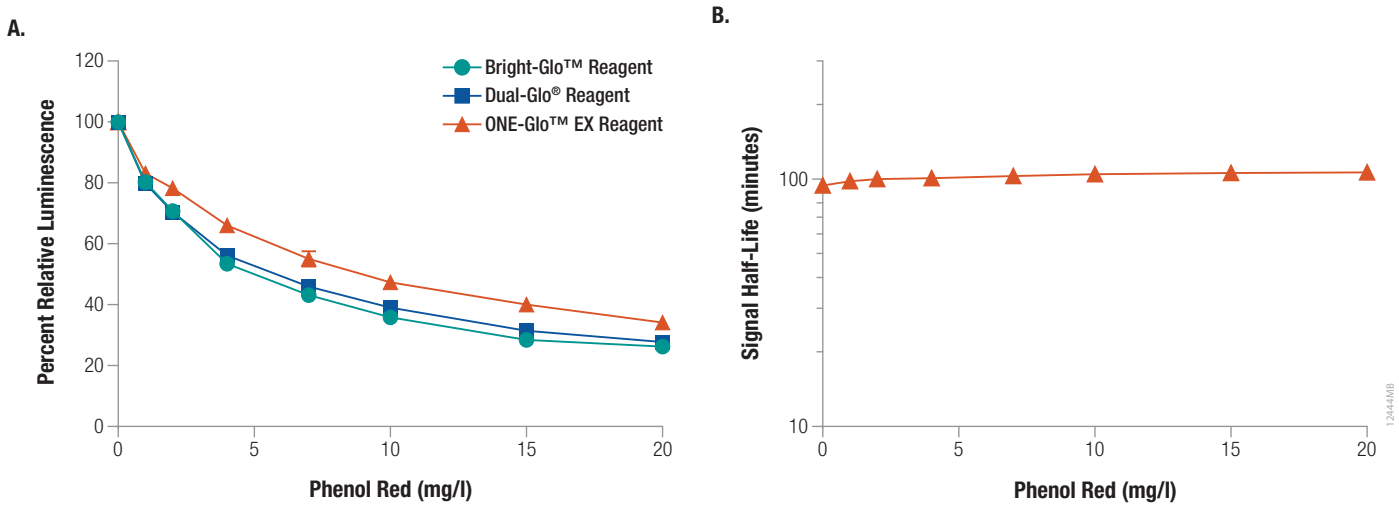

Figure 7. The effect of phenol red on luminescence intensity and stability. Purified firefly luciferase (6.88ng/ml) was diluted into DMEM plus 0.1% Prionex® with varying concentrations of phenol red. Enzyme solutions (80µl per well) were added to 96-well plates, then 80µl of Bright-Glo®, Dual-Glo® Luciferase or ONE-Glo™ EX Reagent was dispensed. Luminescence was measured periodically over 2 hours to calculate the signal stability (half-life); n = 3. **Panel A.** Firefly luminescence relative to no added phenol red. **Panel B.** Signal half-life for ONE-Glo™ EX Reagent.

Temperature

Because the activity of firefly luciferase is temperature sensitive, maintaining a consistent temperature is an important factor in experimental precision. Higher temperature causes higher signal intensity but lower signal stability (Figure 8). Precision can be most easily achieved by performing all experiments at room temperature. The assay reagents should be at room temperature before measuring luminescence, and the culture plate should be equilibrated to room temperature before adding reagents.

The ONE-Glo™ EX Buffer can be stored at room temperature prior to the experiment to eliminate the need for temperature equilibration before use. The heat capacity of the substrate is low; therefore, reconstitution of the substrate with room temperature buffer will produce reagents ready for use. If equilibration is needed to bring reagents to room temperature, incubate reagents in a water bath at room temperature (the water bath should not be set higher than 25°C).

Reaction temperatures can be affected by chilled reagent, culture plates that are too warm, excess heat within luminometers and other factors. If cold reagent is used, luminescence will slowly increase during the experiment as the reagent warms. Some luminometers run at a higher temperature than the ambient environment. To prevent signal gradients across a plate due to uneven warming of a plate during measurement, we recommed equilibrating plates and reagents to the internal temperature of the luminometer (e.g., in a water bath set to the higher-than-ambient temperature of the instrument).

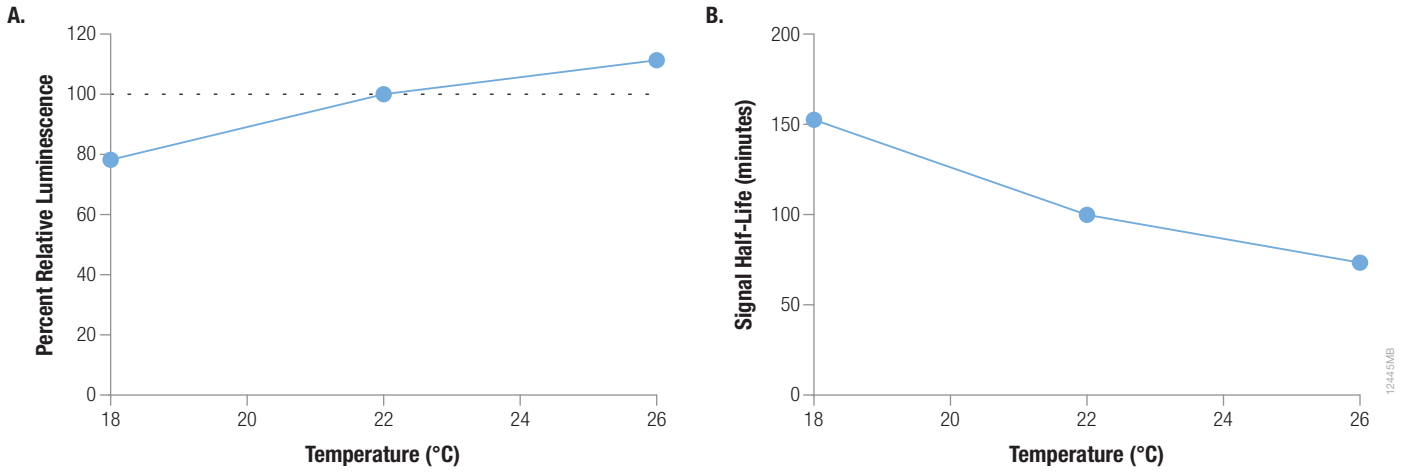

Figure 8. The effect of temperature on luciferase luminescence. Purified firefly luciferase (13.8ng/ml) was diluted into DMEM plus 0.1% Prionex®. Luciferase solution (200µl) was added to 200µl of ONE-Glo™ EX Reagent in a luminometer tube. Luminescence was measured periodically over 2 hours using a Turner Biosystems 20/20^{n} luminometer. The luciferase solutions and reagents were equilibrated at 18, 22 and 26°C prior to mixing and incubated at those same temperatures between measurements. **Panel A.** Luminescence after 3 minutes relative to the value at 22°C. **Panel B.** Signal half-life.

5.C. Reference

1. Fan, F. and Wood, K.V. (2007) Bioluminescent assays for high-throughput screening. *Assay Drug Dev. Technol.* **5**, 127–36.

6. Related Products

To find a firefly luciferase reporter vector for your experiment, visit: **www.promega.com/luciferase-vectors**

Luciferase Assay Systems

Product	Size	Cat.#
ONE-Glo™ Luciferase Assay System*	100ml	E6120
Steady-Glo® Luciferase Assay System*	100ml	E2520
Bright-Glo™ Luciferase Assay System*	100ml	E2620
Dual-Glo® Luciferase Assay System*	100ml	E2940
Dual-Luciferase® Reporter Assay System*	100 assays	E1910
Luciferase Assay System*	100 assays	E1500
Luciferase Assay Reagent	1,000 assays	E1483
Renilla-Glo® Luciferase Assay System*	100ml	E2720
Nano-Glo® Dual-Luciferase® Reporter (NanoDLR™) Assay System*	100ml	N1620
QuantiLum® Recombinant Luciferase*	1mg	E1701

*Additional Sizes Available.

Transfection Reagent

Product	Size	Cat.#
FuGENE® HD Transfection Reagent	1ml	E2311
	5 × 1ml	E2312
FuGENE® 6 Transfection Reagent	1ml	E2691
	0.5ml	E2693
	5 × 1ml	E2692
ViaFect™ Transfection Reagent	0.75ml	E4981
	2 × 0.75ml	E4982
Transfection Carrier DNA	5 × 20µg	E4881

Luminometers

Product	Cat.#
GloMax® Discover System	GM3000
GloMax®-Multi+ Detection System with Instinct™ Software Base Instrument with Shaking*	E8032
GloMax®-Multi+ Detection System with Instinct™ Software Base Instrument with Heating and Shaking*	E9032
GloMax®-Multi Base Instrument*	E7031
GloMax® 96 Microplate Luminometer	E6501
GloMax® 20/20 Luminometer	E5311

*Base instrument must be purchased with luminescence modules (e.g., E8032 and E9032 with E8041 or E7031 with E7041).

[a]Patent Pending.

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

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All prices and specifications are subject to change without prior notice.

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