

# Luciferase Assay System Vendor Comparison

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*Promega's Luciferase Assay System provides an assay method which is substantially improved over conventional methods for both sensitivity and simplicity because constant light intensity is generated. In this article, we compared the Promega Luciferase Assay Reagent to luciferase assay systems from three other manufacturers. This comparison revealed that Promega's Assay System provides greater user convenience and assay stability not found in other commercially available assay systems.*

## Introduction

The traditional firefly luciferase reaction utilizes beetle luciferin, O<sub>2</sub>, and ATP/Mg<sup>2+</sup> to produce a rapid "flash-kinetic" light emission. This flash-kinetic reaction requires the use of an instrument fitted with a dedicated reagent injection device, is difficult to perform reproducibly and limits the sensitivity of the luminescent assay.

Research conducted at Promega led to the development of a novel luciferase assay chemistry incorporating coenzyme A to provide both steady-state luminescent output (signal half-life  $\geq 10$  minutes), and overall light emission that is greatly increased over the conventional luciferase reporter assay. In January of 1991, Promega offered the first available "glow-kinetic" luciferase chemistry as an integrated Luciferase Assay System (Cat.# E1500).

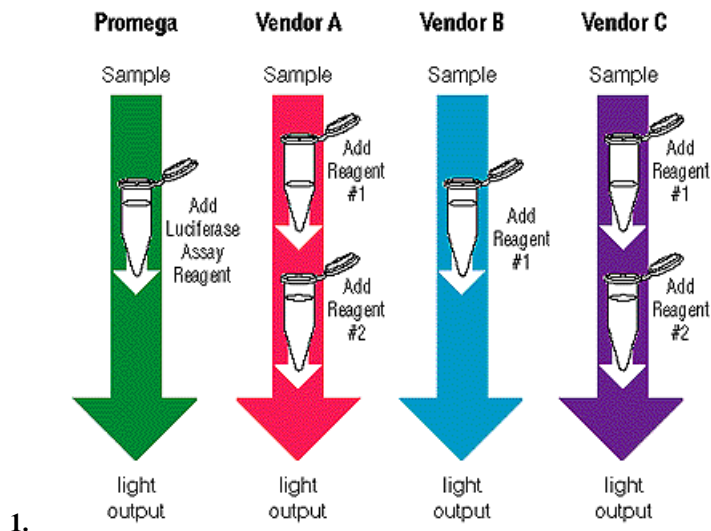
Owing to its reliability, extreme sensitivity and linear dynamic range, as well as its unsurpassed speed and convenience, the firefly luciferase assay is commonly recognized as the system of choice for quantitative reporting of genetic activity. Furthermore, the benefits of the glow-kinetic assay, as introduced by Promega, now make it the standard method for measuring luciferase activity.

The success of the glow-kinetic assay has prompted other vendors to offer similar assay reagents. In this article, we compare the performance and convenience of Promega's Luciferase Assay System to products from three other manufacturers. One additional commercial luciferase assay system is also available, however it was developed to accommodate applications requiring extreme longevity of light emission (signal half-life  $\geq 5$  hours). The very long signal half-life of this vendor's assay is gained at the expense of greatly reduced luciferase activity, and consequently assay sensitivity. We acknowledge the specialized application for which this product is intended, and therefore did not include it in our comparative evaluation.

## Comparison of assay convenience

One of the advantages to using luciferase as a reporter of genetic activity is the convenience and speed of performing the luminescence assay. Therefore, the number of reagents that must be prepared (reconstituted, stored, thawed) and utilized per assay is an important factor in assessing the overall convenience of the various commercially available luciferase assay kits. The necessity of making two sequential reagent additions per assay is cumbersome for users who manually pipet the reagents. Even when using luminometers capable of automated sample and reagent handling, the requirement for two reagent additions per assay translates into reduced assay throughput and increased instrumentation cost.

The reagent additions required to perform each of the four luciferase assays evaluated are presented schematically in [Figure 1](#). The luciferase assay systems of Vendors A and C require the reconstitution of two reagents, and their sequential addition to the lysate sample prior to quantitating luciferase activity. The convenience of adding a single reconstituted assay reagent per sample tube is provided only by the assay kits of Promega and Vendor B.

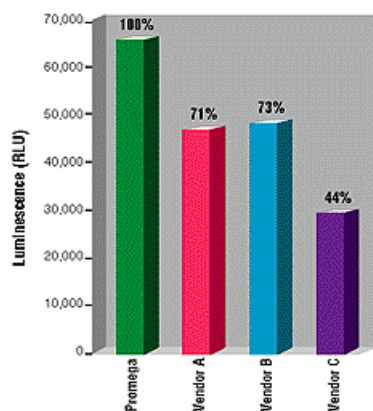


1. **Figure 1. Assay formats of commercially available firefly luciferase reporter assay systems.**

## Comparison of performance characteristics

A meaningful comparison of performance quality between luciferase assay reagents with different chemical formulations was achieved by determining how well each of the assay chemistries supports both absolute activity and catalytic stability of the luciferase reporter enzyme. Thus, a comparative evaluation of each vendor's luciferase assay formulation was performed by measuring the intensity of luminescence reactions catalyzed by a standard concentration of luciferase, as well as the stability of light emission for each reaction over a set assay period.

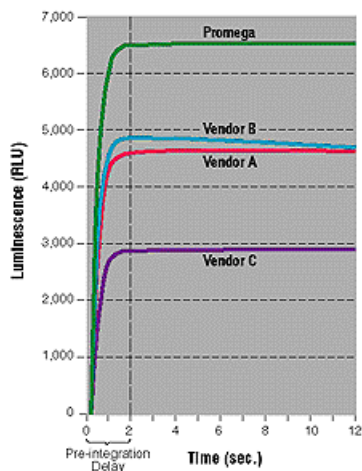
The average absolute luminescence intensities of reactions performed using each vendor's assay kit reagents are presented graphically in [Figure 2](#). Reactions were performed using mock cell lysates containing a standard concentration of luciferase. The mock lysates were prepared using the lysis reagent provided with each vendor's assay kit or, in the absence of a supplied lysis reagent, using Promega's Cell Culture Lysis Reagent (CCLR), as described in the figure legend. Calculated values corresponding to the relative intensities of emitted light are also presented in [Figure 2](#). The luminescence activities of assays performed using the kit reagent(s) of Vendors A, B and C are, respectively, 71%, 73% and 44% that of the total activity measured from the Promega Luciferase Assay Reagent.



1. **Figure 2. Comparative performance of commercially available luciferase assay chemistries.** Luciferase assay reagents were prepared as directed in the technical literature accompanying each assay kit. Mock cell lysates were prepared by adding purified recombinant luciferase (Enliten® Recombinant Luciferase; Cat.# E1701) and Blot Qualified BSA (Cat.# W3841) (final concentrations of 0.2µg/ml and 1 mg/ml, respectively) to the individual cell lysis reagents (1X) supplied with three of the four kits evaluated. Vendor C does not include a cell lysis reagent as part of their assay kit, therefore this vendor's assay reagent, as well as that of Promega, was tested using mock cell lysate prepared with Promega's Cell Culture Lysis Reagent (CCLR; Cat.# E1531). Luminescent reactions were initiated by adding 40µl of the appropriate mock cell lysate to 8 x 50mm luminometer cuvettes containing 200µl of either Promega's or Vendor B's Assay Reagent, or successive 100µl aliquots of Assay Reagents "1" and "2" of Vendor A and C's systems. A Turner Designs Model 20e Luminometer was fitted with a neutral density filter and used to measure luciferase activity. After the elapse of an initial 2 second delay, the intensity of total emitted light was integrated over a 10 second reaction period. Absolute light intensity for each reaction set is presented in terms of Relative Light Units (RLU). Values of percent activity relative to that of Promega's Luciferase Assay System have been calculated and are presented above

each respective bar. The bars represent an average RLU value derived from sufficient sample replicates ( $n \geq 4$ ) to achieve a relative standard deviation of  $\leq 2\%$ .

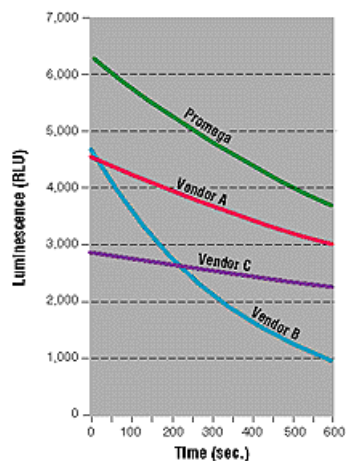
Luciferase activity is typically quantified by either integrating light intensity or counting photon "hits" over a defined time period. Therefore, signal decay over the short duration of the luciferase assay may introduce unforeseen variability to manual users who are not attentive to controlling the elapsed interval between the time the reaction was initiated and the actual start of the assay measurement. As seen by the average signal tracings presented in [Figure 3](#), the luciferase assay reagents offered by Promega, Vendor A and Vendor C show no deviation in signal output over the 10 second measurement period (occurring between 2 and 12 seconds post-initiation of the luciferase reaction). However, the luminescent reaction provided by the luciferase assay system of Vendor B shows significant signal decay ( $> 3.5\%$ ) over the same 10 second assay period. Such rapid signal decay may be particularly troublesome for those researchers using a modified scintillation counter to quantify luciferase assays. As seen previously in [Figure 2](#), the luminescent emission provided by Promega's Luciferase Assay System is significantly higher than that obtained using the other vendors' assay chemistries.



1.

**Figure 3. Stability of light emission over a 10 second period of signal measurement.** Luciferase assays were performed and the data calculated as described in [Figure 2](#).

The tracings presented in [Figure 4](#) show the extended stability of luciferase assays performed using reagents from the four luciferase assay kits under evaluation. Although the assay system of Vendor C provides the lowest initial level of luciferase activity (and therefore, assay sensitivity), its assay reagents are found to provide the greatest long-term stability of emitted luminescence. The immediate onset of signal decay previously observed when using luciferase assay reagent purchased from Vendor B is very dramatic over the 10 minute reaction period. Extended luciferase assays conducted with reagents supplied by Promega and Vendor A produce similar patterns of signal decay.



1.

**Figure 4. Stability of light emission over a 10 minute period of signal measurement.** Luciferase assays were performed and the data calculated as in [Figure 2](#).

## Discussion

The focus of this study was to assess the convenience and overall performance of four commercially available luciferase assay reagents.

Each assay system is offered by its respective manufacturer for the purpose of performing rapid and sensitive quantitation of luciferase reporter activity in prepared cell extracts.

In terms of user convenience, only Promega and Vendor B provide a luciferase assay system comprised of a single reconstituted assay reagent. This feature reduces reagent preparation and handling time, and eliminates the need and cost of an accessory reagent injector for high-throughput instruments.

Using luciferase assay reagents that support maximal luciferase activity is critical because the luminescent intensity of the luciferase-mediated reaction directly impacts the detection sensitivity of the reporter assay. Thus, the performance quality of each luciferase assay system was assessed by determining the absolute luciferase activity it provided as well as the stability of the luminescent signal over time. All luciferase assays were performed using equivalent amounts and concentrations of luciferase enzyme. Therefore, the absolute intensity of each luminescent reaction provides a reliable comparative indicator of the catalytic activity of luciferase in each assay mix. Likewise, the stability of emitted luminescence during the course of a luciferase reaction is indicative of the catalytic stability of the luciferase enzyme in each assay mix. Based on the comparison of luciferase activity presented in [Figure 2](#), Promega's Luciferase Assay System is clearly superior to the luciferase assay reagents offered by other vendors. In terms of stability, 10 second signal tracings demonstrated constant luminescence output from luciferase reactions performed using the kit reagents of Promega, Vendor A and Vendor C. Consequently, users of these kit reagents can be confident that small inconsistencies in elapsed pre-measurement time between sample reactions will not detract from assay precision or accuracy. Only the luciferase reagent manufactured by Vendor B produced significant signal decay over the 10 second assay period.

## Summary

Our analysis of competitive luciferase assay systems reveals that Promega's Luciferase Assay Reagent provides user convenience and assay stability not found in other commercially available assay systems. Of greatest significance is the demonstration of unsurpassed luciferase activity, and therefore assay sensitivity, provided by Promega's Luciferase Assay System.

## Ordering Information

Product	Cat. #
----- Luciferase Assay System -----	E1500
Luciferase Assay System with Reporter Lysis Buffer -----	E4030
Luciferase Assay Reagent 10-Pack -----	E1501
Luciferase Assay Reagent -----	E1483