

A More Dynamic DLR™ Assay

Increased *Renilla* Luciferase Sensitivity in the Dual-Luciferase® Reporter Assay System

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Abstract

The Dual-Luciferase® Reporter Assay System is a robust, simple and very sensitive assay system used to measure two luciferase activities simultaneously from a single sample. Proprietary technology has increased *Renilla* luciferase sensitivity by lowering the enzyme-independent luminescence in the *Renilla* luciferase portion of the assay.

New technology has improved the DLR™ Assay System, resulting in even greater assay sensitivity.

Introduction

The most valuable reporter assays are those that provide the most information and the greatest sensitivity in a simple, reliable format. Systems that individually measure multiple reporter genes are thus the most sought after assays.

In 1996, the Dual-Luciferase® Reporter (DLR™) Assay System^(a,b,c) was one of the first assays to provide information from two reporter genes in a single sample and is still one of the most sensitive yet simplest assays available. New technology has improved the DLR™ Assay System, resulting in even greater assay sensitivity.

The DLR™ Assay System is based on measurement of both firefly and *Renilla* luciferase activities. The chemistry for each of these reactions is completely separate, with molecular oxygen being the only

component common to the two reactions (Figure 1). The independence of the two chemistries permits initiation of the firefly luciferase luminescence by addition of Luciferase Assay Reagent II^(a,c), with subsequent post-measurement quenching using Stop & Glo® Reagent. Stop & Glo® Reagent, while quenching firefly luciferase, also initiates the *Renilla* luciferase luminescence reaction. Thus the luminescence from the two luciferases can be measured independently.

Firefly luciferase is an extremely sensitive reporter since (i) firefly luciferase reagents do not generate background luminescence and (ii) firefly luciferase is not endogenously expressed in mammalian cells. *Renilla* luciferase has traditionally been considered less sensitive than firefly luciferase because coelenterazine, the substrate for *Renilla* luciferase, can undergo enzyme-independent oxygenation producing light referred to as autoluminescence.

The new Dual-Luciferase® Reporter Assay System has lower autoluminescence than the original DLR™ System formulation. This lowered autoluminescence permits more sensitive measurement of, and thus detection of, smaller amounts of *Renilla* luciferase from a cell lysate sample. Autoluminescence increases in the presence of detergents and other hydrophobic environments (1). Although Passive Lysis Buffer (supplied with the DLR™ System) is specifically formulated to lyse cells with minimal autoluminescence in the *Renilla* luciferase portion of the assay, PLB contains detergent in order to lyse cells. Proprietary technology developed for the Dual-Glo™

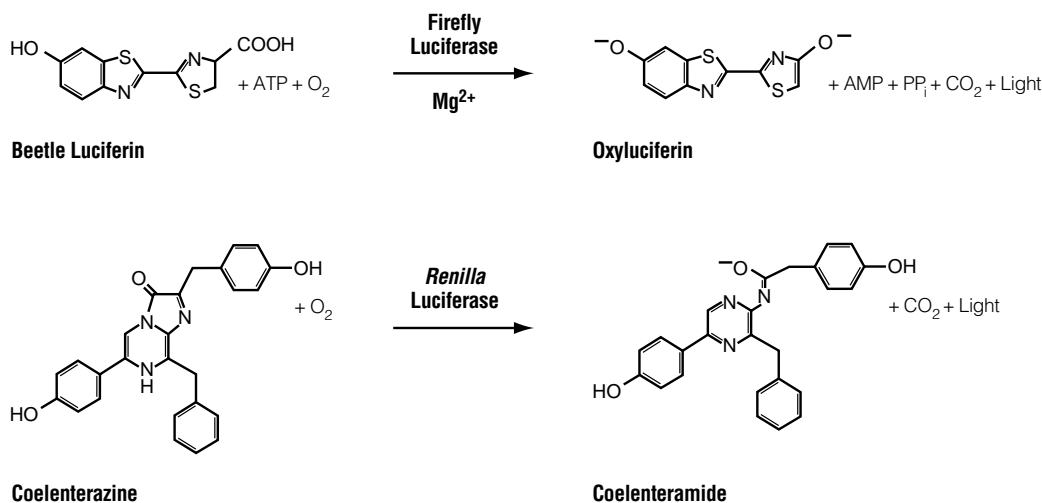


Figure 1. Firefly and *Renilla* luciferase reactions utilize different substrates.

Increased *Renilla* Luciferase Sensitivity... continued

Assay System^(a,b,c) (Cat.# E2920) and the *Renilla* Luciferase Assay System^(c,d) (Cat.# E2810) has resulted in selective quenching of autoluminescence without decreasing the enzyme-dependent luminescence. This same technology is now incorporated into the new Stop & Glo[®] Reagent to reduce the autoluminescence that previously persisted in the presence of Passive Lysis Buffer. By decreasing the autoluminescence in the reaction environment, the sensitivity of the assay has been improved by approximately 5- to 10-fold (Figure 2).

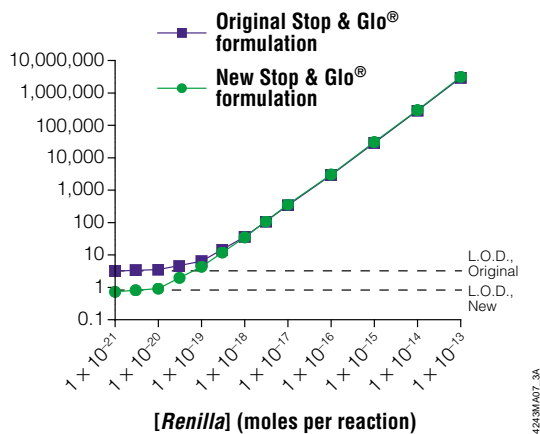


Figure 2. The new formulation of Stop & Glo[®] Reagent increases *Renilla* luciferase assay sensitivity by approximately 5- to 10-fold. Purified *Renilla* luciferase-GST fusion protein (Chemicon) was diluted into 1X Passive Lysis Buffer containing 0.1% gelatin (w:v) and monitored with the Dual-Luciferase[®] Reporter Assay System. The same Luciferase Assay Reagent II was used for the data sets, one containing new (E640 + E641) and one the original (E197 + E463 + E193) Stop & Glo[®] Reagents. The two data sets were run simultaneously, alternating between samples containing the new and the original formulations. Luminescence was measured on a Turner 20e luminometer, integrating each sample for 10 seconds after a 2-second delay. For data points where *Renilla* luciferase $< 1 \times 10^{-18}$ moles per reaction, n=5; for data points where *Renilla* luciferase $> 1 \times 10^{-18}$ moles per reaction, n=3. Data shown is the average of n samples with standard deviations. Level of detection (L.O.D.) is background luminescence plus three standard deviations of background.

The improved formulation affects autoluminescence but not luminescence, leaving the intensity and kinetics of the *Renilla* luminescence generally unaffected (Figures 2 and 3). Similarly, the new formulation does not affect the other functions of the Stop & Glo[®] Reagent. For instance, quenching of the firefly luciferase reaction is also unaffected by the change (Figure 4). To make the DLR[™] Assay easier to use, Stop & Glo[®] Substrate, previously provided lyophilized with a separate solvent for reconstitution, is now provided in solution in a tube with a screw cap for easy closing and that prevents evaporation during storage.

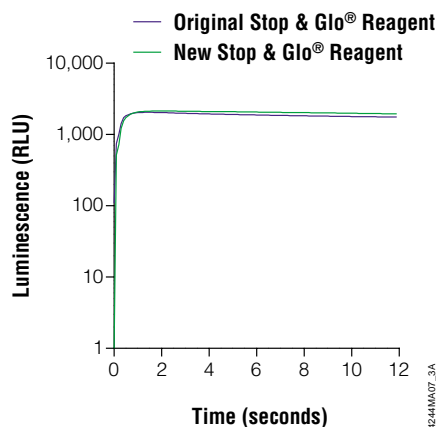


Figure 3. The new formulation of the Stop & Glo[®] Reagent generates similar kinetics for *Renilla* luciferase luminescence. Purified *Renilla* luciferase-GST fusion (Chemicon) was diluted to 1×10^{-15} moles per reaction in 1X PLB + 0.1% gelatin. The reactions were performed as instructed in the *Dual-Luciferase[®] Reporter Assay System Technical Manual*, #TM040: 20µl enzyme samples were combined with 100µl of Luciferase Assay Reagent II and 100µl of the original or new Stop & Glo[®] Reagent formulation. Luminescence was captured as a data stream for 12 seconds after the reaction was initiated by Stop & Glo[®] Reagent addition. Data shown are the average of 3 samples. The CV for a 10-second integration of each data point shown was <4.4%.

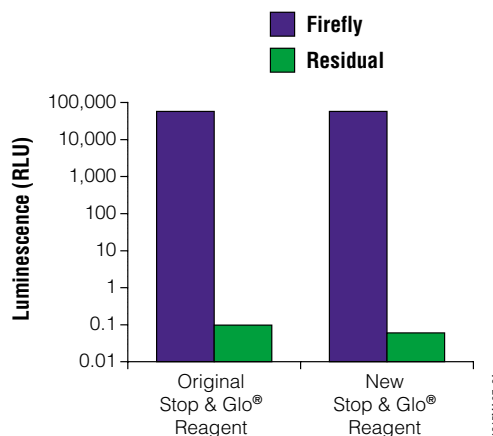


Figure 4. The new formulation of the Stop & Glo[®] Reagent quenches firefly luciferase by at least 100,000-fold. QuantiLum[®] Recombinant Luciferase^(e) (Cat.# E1701) at 4.4×10^{-15} moles per 20µl sample reaction was combined with 100µl of Luciferase Assay Reagent II, and the luminescence was integrated over 10 seconds after a 2-second delay. Stop & Glo[®] Buffer without Stop & Glo[®] Substrate was then added (100µl), samples were vortexed and the residual luminescence was measured. Firefly luminescence decreased by greater than 500,000-fold when using either the new or original Stop & Glo[®] formulations.

Summary

The DLR[™] Assay System has always been a robust, simple and very sensitive assay system used to measure two luciferases from a single sample. Promega has improved this assay by increasing *Renilla* luciferase sensitivity. Decreasing the autoluminescence in the *Renilla* luciferase portion of the assay has increased *Renilla* luciferase sensitivity by approximately 5- to 10-fold without adversely affecting the other characteristics of the assay system.

Reference

1. *Renilla Luciferase Assay System Technical Manual*, #TM055, Promega Corporation.

Protocol

- ◆ *Dual-Luciferase® Reporter Assay System Technical Manual* #TM040, Promega Corporation.
(www.promega.com/tbs/tm040/tm040.html)
- ◆ *Dual-Luciferase® Reporter 1000 Assay System Technical Manual* #TM046, Promega Corporation.
(www.promega.com/tbs/tm046/tm046.html)

Ordering Information

Product	Size	Cat.#
Dual-Luciferase® Reporter Assay System ^(a,b,c)	100 assays	E1910
Dual-Luciferase® Reporter Assay System 10-pack ^(a,b,c)	1,000 assays	E1960
Dual-Luciferase® Reporter 1000 Assay System ^(a,b,c)	1,000 assays	E1980
Passive Lysis 5X Buffer	30ml	E1941
QuantiLum® Recombinant Luciferase ^(e)	1mg	E1701

^(a)U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289 and 5,814,471, Australian Pat. No. 649289 and other patents and patents pending.

^(b)U.S. Pat. No. 5,744,320, Australian Pat. No. 721172 and other patents and patents pending.

^(c)Certain applications of this product may require licenses from others.

^(d)Patent Pending.

^(e)The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673. If any product contains recombinant *Coleoptera* luciferase nucleic acid capable of producing light when expressed, a license (from Promega for research reagent products and from The Regents of the University of California for all other fields) is needed for any commercial sale of nucleic acid contained within or derived from this product.

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