

Luciferase Assay System

INSTRUCTIONS FOR USE OF PRODUCTS E1483, E1500, E1501, E1531, E4030, E4530 AND E4550.

Luciferase Assay System

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Please visit the web site to verify that you are using the most current version of this Technical Bulletin. Please contact Promega Technical Services if you have questions on use of this system. E-mail: techserv@promega.com.

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1. Description

Genetic reporters are used commonly in cell biology to study gene expression and other cellular events coupled to gene expression, such as receptor activity, intracellular signal transduction, mRNA processing, protein folding and protein:protein interactions (1,2). Firefly luciferase is widely used as a reporter for the following reasons:

- Reporter activity is available immediately upon translation since the protein does not require post-translational processing (3,4).
- The assay is very sensitive because its light production has the highest quantum efficiency known for any chemiluminescent reaction (5), and no background luminescence is found in the host cells or the assay chemistry.
- The assay is rapid, requiring only a few seconds per sample.

The Luciferase Assay System^(a,b,c) is substantially improved over conventional assay methods in both sensitivity and simplicity (2,6–8). Light is produced by converting the chemical energy of luciferin oxidation through an electron transition, forming the product molecule oxyluciferin. Firefly luciferase, a monomeric 61kDa protein, catalyzes luciferin oxidation using ATP•Mg²⁺ as a cosubstrate (Figure 1). In the conventional assay for luciferase, a flash of light is generated that decays rapidly after the enzyme and substrates are combined. The Luciferase Assay System incorporates coenzyme A (CoA) for improved kinetics (9), allowing greater enzymatic turnover and resulting in increased light intensity that is nearly constant for at least 1 minute (Figure 2). The Luciferase Assay System yields linear results over at least eight orders of magnitude. Less than 10⁻²⁰ moles of luciferase have been detected under optimal conditions (2). Generally, 100-fold greater sensitivity can be achieved over the chloramphenicol acetyltransferase (CAT) assay (1).

The Luciferase Assay System was developed for reporter quantitation in mammalian cells. The Luciferase Assay System (Cat.# E1500), provided with Cell Culture Lysis Reagent (CCLR), can also be used for reporter quantitation in plant and bacterial cells (see Section 3.E); however, the Luciferase Assay System with Reporter Lysis Buffer (Cat.# E4030) is not suitable for these applications.

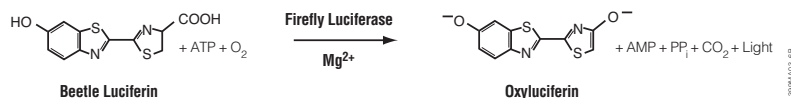


Figure 1. Bioluminescent reaction catalyzed by firefly luciferase.

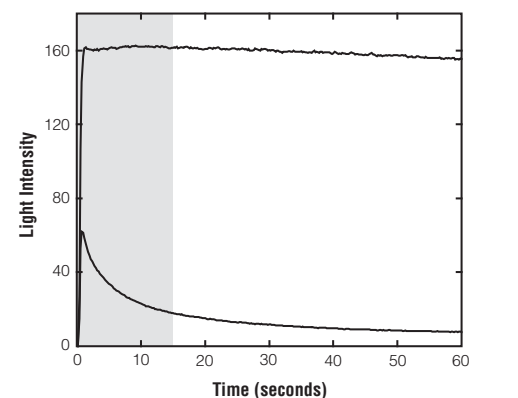


Figure 2. Comparison of the Promega Luciferase Assay System to the conventional luciferase assay method. NIH3T3 cells expressing the luciferase gene from Rous sarcoma virus were lysed with 1X Cell Culture Lysis Reagent 48 hours after infection. The shaded area represents the light typically lost in measurements where the cell lysate is mixed with substrate prior to light detection (e.g., scintillation counting). In the conventional assay, this is 50% of the total luminescence in a 1-minute measurement.

The Luciferase Reporter 1000 Assay System (Cat.# E4550) was designed to meet the needs of users who perform a large number of assays, particularly in 96-well plates. The system contains sufficient reagents to perform 1,000 luciferase assays (100µl per assay). For users working with transformed cells, a cell lysis buffer will be needed for sample preparation prior to luciferase measurement (see Section 3). The lysis buffer must be purchased separately.

The Luciferase Assay System is generally used with a lysis buffer and Luciferase Assay Reagent. Luciferase Assay Reagent and its preparation are described in Section 3.B. The three lysis buffers are described in Section 3.C, and Table 1 recommends the appropriate lysis buffer for use with a particular cell type.

2. Product Components and Storage Conditions

Product	Size	Cat.#
Luciferase Assay System	100 assays	E1500

Each system contains sufficient reagents for 100 standard assays. Includes:

- 1 vial Luciferase Assay Substrate (lyophilized)
- 10ml Luciferase Assay Buffer
- 30ml Luciferase Cell Culture Lysis Reagent, 5X

Product	Size	Cat.#
Luciferase Assay System 10-Pack	1,000 assays	E1501

Contains sufficient reagents for 1,000 standard assays. Includes:

- 10 vials Luciferase Assay Substrate (lyophilized)
- 10 × 10ml Luciferase Assay Buffer

Product	Size	Cat.#
Luciferase Assay System with Reporter Lysis Buffer	100 assays	E4030

Each system contains sufficient reagents for 100 standard assays. Includes:

- 1 vial Luciferase Assay Substrate (lyophilized)
- 10ml Luciferase Assay Buffer
- 30ml Reporter Lysis 5X Buffer

Product	Size	Cat.#
Luciferase Assay System Freezer Pack	1,000 assays	E4530

Each system contains sufficient reagents for 1,000 standard assays. Includes:

- 10 vials Luciferase Assay Substrate (lyophilized)
- 10 × 10ml Luciferase Assay Buffer
- 10 vials Reporter Lysis 5X Buffer (30ml/vial)

Product	Size	Cat.#
Luciferase Reporter 1000 Assay System	1,000 assays	E4550

Each system contains sufficient reagents for 1,000 standard assays. Includes:

- 1 vial Luciferase Assay Substrate (lyophilized)
- 105ml Luciferase Assay Buffer

Product	Size	Cat.#
Luciferase Assay Reagent	1,000 assays	E1483

Contains sufficient reagent for 1,000 standard assays. Includes:

- 100ml Luciferase Assay Reagent

Product	Size	Cat.#
Luciferase Cell Culture Lysis 5X Reagent	30ml	E1531

Includes:

- 30ml Luciferase Cell Culture Lysis 5X Reagent

Storage and Stability: Luciferase Assay Reagent can be purchased ready to use (Cat.# E1483) or prepared by reconstituting **Luciferase Assay Substrate** with **Luciferase Assay Buffer**. See the product label for the expiration date of Cat.# E1483. Reconstituted Luciferase Assay Reagent (Luciferase Assay Substrate + Luciferase Assay Buffer) should be stored in aliquots at -20°C for up to 1 month or at -70°C for up to 1 year. **Do not** store Luciferase Assay Reagent with dry ice. Thaw Luciferase Assay Reagent at temperatures below 25°C and mix well before use.

Store Luciferase Assay Substrate in the dark. Reporter Lysis Buffer may be stored at room temperature and should be stored away from direct sunlight. Luciferase Cell Culture Lysis Reagent should be stored at -20°C.

3. Preparations Prior to Performing the Luciferase Assay

Before beginning a luciferase assay for the first time, prepare the Luciferase Assay Reagent (Section 3.B) and the lysis buffer (Sections 3.C-D). Important light-detection considerations are noted in Section 3.A. In addition, Section 5 provides information on optimizing light intensity and choice of light detection instrumentation.

3.A. Determining the Linear Range of Light Detection

It is important to determine the linear range of light detection for your luminometer before performing an experiment, because luminometers can experience signal saturation at high light intensities. To produce a standard curve of light units versus relative enzyme concentration, make serial dilutions of luciferase (either purified luciferase or cell culture lysate) in any 1X lysis buffer supplemented with 1mg/ml BSA. The addition of BSA is necessary to ensure that luciferase is not lost from solution by adsorption. Recombinant firefly luciferase is available from Promega (QuantiLum® Recombinant Luciferase, Cat.# E1701).

3.B. Luciferase Assay Reagent Preparation

To prepare the **Luciferase Assay Reagent**, add **Luciferase Assay Buffer** (105ml for Cat.# E4550; 10ml for other systems) to the vial containing the lyophilized **Luciferase Assay Substrate**. **Note:** Luciferase Assay Reagent is also available premixed (Cat.# E1483). Avoid exposure of the Luciferase Assay Reagent to multiple freeze-thaw cycles by dispensing the reconstituted reagent into working aliquots. Store any unused Luciferase Assay Reagent at -70°C. Equilibrate Luciferase Assay Reagent to room temperature before each use. Each reaction requires 100µl of the Luciferase Assay Reagent to initiate enzyme activity.

3.C. Lysis Buffers

Promega has three lysis buffers that can be used to prepare cell lysates containing luciferase (see Table 1). Luciferase Cell Culture Lysis Reagent (CCLR) provides efficient lysis within minutes. Reporter Lysis Buffer (RLB) is a mild lysis agent and requires a single freeze-thaw cycle to achieve complete cell lysis. Passive Lysis Buffer (PLB; Cat.# E1941) will passively lyse cells without the requirement of a freeze-thaw cycle. However, lysis efficiency is dependent upon the cell type and needs to be determined for those cells that are resistant to passive lysis. PLB contains an anti-foam agent, which prevents excessive bubbling of the sample when the reagent is delivered with force by an automated dispenser. The absence of bubble formation may result in more consistent detection of light output and prevents instrument contamination.

Note: For applications involving the coexpression of firefly luciferase with a second reporter gene, we recommend preparing cell lysates with either RLB or PLB.

Table 1. Recommended Lysis Buffers for Various Sample Types.

Sample/Cell Type	Lysis Buffer
adherent mammalian cells	CCLR, RLB, PLB
nonadherent mammalian cells	CCLR, RLB, PLB
bacterial cells ^{1,2}	CCLR
plant cells ¹	CCLR
tissue homogenates	CCLR, RLB

¹Section 6 contains information on the CCLR formulation (lysis mix) recommended for bacterial cell lysis.

²RLB has not been qualified for use with plant or bacterial cells.

3.D. Protocol for Preparing Cell Lysates

1. Add 4 volumes of water to 1 volume of 5X lysis buffer. Equilibrate 1X lysis buffer to room temperature before use.
2. Carefully remove the growth medium from cells to be assayed. Rinse cells with PBS (see Section 6), being careful to not dislodge attached cells. Remove as much of the PBS rinse as possible.
3. Add enough 1X lysis buffer (CCLR, RLB or PLB) to cover the cells (e.g., 400µl/60mm culture dish, 900µl/100mm culture dish or 20µl per well of a 96-well plate). If using RLB, perform a single freeze-thaw cycle to ensure complete lysis. **For 96-well plates, proceed to Section 4. For culture dishes, continue to Step 4.**
4. Rock culture dishes several times to ensure complete coverage of the cells with lysis buffer. Scrape attached cells from the dish. Transfer cells and all liquid to a microcentrifuge tube. Place the tube on ice.
5. Vortex the microcentrifuge tube 10–15 seconds, then centrifuge at 12,000 × g for 15 seconds (at room temperature) or up to 2 minutes (at 4°C). Transfer the supernatant to a new tube.
6. Store the supernatant/cell lysate at -70°C or proceed to Section 4.

3.E. Protocol for Plant and Bacterial Cell Lysates and Tissue Homogenates

1. For plant tissue, quick-freeze in liquid nitrogen, grind the frozen tissue to a powder and resuspend at room temperature in 1X CCLR with further homogenization. Remove the debris after cell lysis by a brief centrifugation. Assay the supernatant using standard assay conditions (Section 4).
2. For bacteria, mix 40µl of nontransformed cells (carrier cells) with 50µl of a transformed culture. Add 10µl of 1M K₂HPO₄ (pH 7.8), 20mM EDTA. Quick-freeze the mixture on dry ice, then bring the cells to room temperature by placing the tube in a room temperature water bath. Add 300µl of freshly prepared lysis mix (Section 6). Mix and incubate the cells for 10 minutes at room temperature. To assay the lysate, proceed to Section 4.
3. A protocol for the use of tissue homogenates with the Luciferase Assay System can be found in reference 10.

4. Luciferase Assay Protocol

Material to Be Supplied by the User

- opaque multiwell plates or luminometer tubes

The following procedures (Section 4.A–C) are optimized for mammalian cells grown in culture and may also be used with bacterial and plant cell lysates or tissue homogenates as prepared in Section 3.E. The Luciferase Assay System may be used with manual luminometers (those without reagent injectors) or with luminometers that have injectors (either single-tube or 96-well plate types).

Note: The Luciferase Assay Reagent and samples should be at ambient temperature prior to performing a luciferase assay (see Section 5.A).

4.A. Protocol for Manual Luminometers

1. Dispense 100µl of the Luciferase Assay Reagent into luminometer tubes, one tube per sample.
2. Program the luminometer to perform a 2-second measurement delay followed by a 10-second measurement read for luciferase activity. The read time may be shortened if sufficient light is produced.

Note: When using shorter assay times, validate the luminometer over that time period to ensure that readings are taken at a flat portion of the signal curve.

3. Add 20µl of cell lysate to a luminometer tube containing the Luciferase Assay Reagent. Mix by pipetting 2–3 times or vortex briefly.
4. Place the tube in the luminometer and initiate reading.
5. If the luminometer is not connected to a printer or computer, record the reading.

4.B. Protocol for Single-Tube Luminometers with Injectors

1. Prime the luminometer injector at least three times with Luciferase Assay Reagent or as recommended in the owner's manual.
2. Dispense 20µl of cell lysate or test sample into a luminometer tube.
3. Program the luminometer to perform a 2-second measurement delay followed by a 10-second measurement read for luciferase activity. The read time may be decreased if sufficient light is produced.
4. Place the tube in the luminometer and initiate reading by injecting 100µl of Luciferase Assay Reagent into the tube.
5. If the luminometer is not connected to a printer or computer, record the reading.

4.C. Protocol for Plate-Reading Luminometers

1. Program the luminometer for the appropriate delay and measurement times.
2. Place the plate, containing 20µl of cell lysate per well, into the luminometer with injector. The injector adds 100µl of Luciferase Assay Reagent per well, then the well is read immediately. The plate is advanced to the next well for a repeat of the inject-then-read process.
3. Measure the light produced for a period of 10 seconds. The light intensity of the reaction is nearly constant for about 1 minute and then decays slowly, with a half-life of approximately 10 minutes. The typical delay time is 2 seconds, and the typical read time is 10 seconds. The assay time may be shortened significantly to decrease the total read time if sufficient light is produced. **For example**, the total read time for all samples in a 96-well plate can be less than 5 minutes.

5. General Considerations

5.A. Optimization of Light Intensity

Light intensity is a measure of the rate of catalysis by luciferase and is therefore dependent upon temperature. The optimum temperature for luciferase activity is approximately room temperature (20–25°C). It is important that the Luciferase Assay Reagent be fully equilibrated to room temperature before beginning measurements. To ensure temperature equilibration, place a thawed aliquot of the Luciferase Assay Reagent in a sealed tube into a water bath maintained at ambient temperature, and equilibrate for at least 30 minutes.

The sample to be assayed should also be at ambient temperature. Generally, luciferase activity is stable for several hours at room temperature in 1X Luciferase Cell Culture Lysis Reagent (Cat.# E1531), Reporter Lysis Buffer (Cat.# E3971) or Passive Lysis Buffer (Cat.# E1941). If specific circumstances make ambient temperature unacceptable, the sample may be left on ice for up to 12 hours. Assay of a cold sample (0–4°C) using standard assay volumes (see Section 4) will result in a 5–10% decrease in enzyme activity.

Notes:

For applications involving the coexpression of firefly luciferase with a second reporter gene, we recommend preparing cell lysates with either RLB or PLB.

Cell lysates prepared using CCLR (Luciferase Cell Culture Lysis Reagent) will not yield optimal results when assaying for CAT, β -galactosidase or *Renilla* luciferase coreporter activities. CAT is partially inhibited by the Triton® X-100 component of CCLR (11). Although β -galactosidase is not directly inhibited by the high detergent concentration of CCLR, a precipitate may form upon mixing β -Galactosidase Assay Buffer with cell lysates prepared using this lysis buffer. The composition of CCLR and RLB significantly inhibits *Renilla* luciferase activity and also contributes excessive levels of coelenterazine auto-luminescence (12). Furthermore, the high concentration of detergent and dithiothreitol (DTT) in CCLR precludes the use of most protein determination assays to quantify total protein in cell lysates prepared with CCLR.

5.B. Instrumentation

Either a luminometer or a scintillation counter can be used for quantitation with the Luciferase Assay System. (There is usually insufficient light output for qualitative visual detection.) A luminometer can measure as little as 10^{-20} moles (0.001pg) of luciferase, whereas a scintillation counter typically has a less sensitive detection limit. However, the limits of sensitivity may vary depending upon the particular instrument used. The assay should be linear in some portion of the detection range of the instrument. Please consult your instrument operator's manual for general operating instructions.

Luminometers

The most convenient method for performing a large number of luciferase assays is to use a luminometer capable of processing a multiwell plate. The light intensity of the assay and the effective linear range is proportional to luciferase concentrations in the range of 10^{-20} to 10^{-13} moles. However, the limits of sensitivity may vary, depending upon the particular instrument used. The limits should be verified on each instrument before analysis of experimental samples (see Section 3.A).

The GloMax™ 96 Microplate Luminometer is an easy-to-use, highly sensitive microplate luminometer with a broad dynamic range. It features two optional injectors and reads both glow- and flash-luminescent reactions in 96-well plates. Its superior sensitivity and dynamic range makes the GloMax™ 96 Microplate Luminometer an ideal laboratory instrument for a wide range of luminescent assays, including Promega bioluminescent reporter, cell-based and biochemical assays.

The GloMax™ 20/20 Luminometer is an ultrasensitive, compact laboratory instrument for measuring luminescent materials. Specifically, the GloMax™ 20/20 Luminometer accommodates measurement of firefly and *Renilla* luciferase for genetic reporter studies and ATP-based biomass analysis and so is an ideal platform for reading Promega bioluminescent assays.

Scintillation Counters

Ideally, the coincidence circuit of the scintillation counter should be turned off. Usually, this is achieved through an option of the programming menu or by a switch within the instrument. If the circuit cannot be turned off, a linear relationship between luciferase concentration and cpm still can be produced by calculating the square root of measured counts per minute (cpm) minus background cpm (i.e., $[\text{sample} - \text{background}]^{1/2}$). To measure background cpm, add Luciferase Assay Reagent to lysis buffer without cells or to a lysate of nontransfected cells.

The sample may be placed directly in the scintillation vial if it completely covers the bottom of the vial (clear or translucent vials are acceptable). Do not add scintillant, because it will inactivate luciferase. Alternatively, place the sample in a microcentrifuge tube, and then place the tube in the scintillation vial. To ensure consistency when working with multiple samples, place each microcentrifuge tube at the same relative position within the scintillation vial.

For consistency in measuring luciferase activity, use the scintillation counter in manual mode. Initiate each sample reaction immediately before measurement, and read the samples one at a time. Because the enzymatic reaction produces light at all wavelengths, read the samples with all channels open (open window). To reduce background counts, it may be necessary to wait 10–30 seconds before counting. Read individual samples for 1–5 minutes.

5.C. Firefly Luciferase Reporter Vectors

The pGL4 Luciferase Reporter Vectors are the next generation of reporter gene vectors optimized for expression in mammalian cells. Numerous configurations of pGL4 Vectors are available, including those with the synthetic firefly *luc2* (*Photinus pyralis*) and *Renilla hRluc* (*Renilla reniformis*) genes, which have been codon optimized for more efficient expression in mammalian cells. Furthermore, both the reporter genes and the vector backbone, including the ampicillin (Amp^r) gene and mammalian selectable marker genes for hygromycin (Hyg^r), neomycin (Neo^r) and puromycin (Puro^r), have been engineered to reduce the number of consensus transcription factor binding sites, reducing background and the risk of anomalous transcription.

The pGL4 Vector backbone is provided with either the *luc2* or *hRluc* genes and, in certain vectors, one or both of two Rapid Response™ reporter genes. The proteins encoded by these Rapid Response™ luciferase genes respond more quickly and with greater magnitude to changes in transcriptional activity than their more stable counterparts.

For more information on advantages of and improvements made to the pGL4 series of vectors, please visit: www.promega.com/pgl4/ or see the *pGL4 Luciferase Reporters Technical Manual #TM259*.

6. Composition of Buffers and Solutions

PBS buffer (Mg²⁺- and Ca²⁺-free)

137mM	NaCl
2.7mM	KCl
4.3mM	Na ₂ HPO ₄
1.4mM	KH ₂ PO ₄

The final pH should be 7.4.

lysozyme (5mg/ml)

Add 1 volume of 1M K₂HPO₄ (pH 7.8), 20mM EDTA to 9 volumes of water. Add lysozyme to a final concentration of 5mg/ml. Vortex until the lysozyme dissolves. Prepare fresh for each use.

Luciferase Cell Culture

Lysis Reagent, 1X

25mM	Tris-phosphate (pH 7.8)
2mM	DTT
2mM	1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid
10%	glycerol
1%	Triton® X-100

lysis mix

1X	CCLR
1.25mg/ml	lysozyme
2.5mg/ml	BSA

Add water to desired volume. Prepare fresh for each use.

7. Related Products

Luciferase Assay Systems and Reagents

Product	Size	Cat.#
Reporter Lysis 5X Buffer	30ml	E3971
Luciferase Cell Culture Lysis 5X Reagent	30ml	E1531
Passive Lysis 5X Buffer	30ml	E1941
Beetle Luciferin, Potassium Salt	5mg*	E1601
QuantiLum® Recombinant Luciferase	1mg*	E1701

*Available in additional sizes.

Product	Size	Cat.#
Steady-Glo® Luciferase Assay System	10ml*	E2510
Bright-Glo™ Luciferase Assay System	10ml*	E2610
Dual-Glo™ Luciferase Assay System	10ml*	E2920

*Available in additional sizes.

Dual-Luciferase® Reporter Assay Systems

Product	Size	Cat.#
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

Luminometers (Single Sample)

Product	Cat.#
GloMax™ 96 Microplate Luminometer	E6501
GloMax™ 96 Microplate Luminometer with Single Injector	E6511
GloMax™ 96 Microplate Luminometer with Dual Injectors	E6521
GloMax™ 20/20 Luminometer	E5311
GloMax™ 20/20 Luminometer with Single Auto-Injector	E5321
GloMax™ 20/20 Luminometer with Dual Auto-Injector	E5331

Plasmid DNA Purification System

Product	Size	Cat.#
PureYield™ Plasmid Midiprep System	25 preps	A2492
	100 preps	A2495

pGL4 Luciferase Reporter Vectors

Please visit www.promega.com to see a complete listing of our reporter vectors.

Vector	Multiple Cloning Region	Reporter Gene	Protein Degradation Sequence	Reporter Gene Promoter	Mammalian Selectable Marker	Cat.#
pGL4.10[luc2]	Yes	luc2 ^A	No	No	No	E6651
pGL4.11[luc2P]	Yes	"	hPEST	No	No	E6661
pGL4.12[luc2CP]	Yes	"	hCL1-hPEST	No	No	E6671
pGL4.13[luc2/SV40]	No	"	No	SV40	No	E6681
pGL4.14[luc2/Hygro]	Yes	"	No	No	Hygro	E6691
pGL4.15[luc2P/Hygro]	Yes	"	hPEST	No	Hygro	E6701
pGL4.16[luc2CP/Hygro]	Yes	"	hCL1-hPEST	No	Hygro	E6711
pGL4.17[luc2/Neo]	Yes	"	No	No	Neo	E6721
pGL4.18[luc2P/Neo]	Yes	"	hPEST	No	Neo	E6731
pGL4.19[luc2CP/Neo]	Yes	"	hCL1-hPEST	No	Neo	E6741
pGL4.20[luc2/Puro]	Yes	"	No	No	Puro	E6751
pGL4.21[luc2P/Puro]	Yes	"	hPEST	No	Puro	E6761
pGL4.22[luc2CP/Puro]	Yes	"	hCL1-hPEST	No	Puro	E6771
pGL4.70[hRluc]	Yes	hRluc ^B	No	No	No	E6881
pGL4.71[hRlucP]	Yes	"	hPEST	No	No	E6891
pGL4.72[hRlucCP]	Yes	"	hCL1-hPEST	No	No	E6901
pGL4.73[hRluc/SV40]	No	"	No	SV40	No	E6911
pGL4.74[hRluc/TK]	No	"	No	HSV-TK	No	E6921
pGL4.75[hRluc/CMV]	No	"	No	CMV	No	E6931
pGL4.76[hRluc/Hygro]	Yes	"	No	No	Hygro	E6941
pGL4.77[hRlucP/Hygro]	Yes	"	hPEST	No	Hygro	E6951
pGL4.78[hRlucCP/Hygro]	Yes	"	hCL1-hPEST	No	Hygro	E6961
pGL4.79[hRluc/Neo]	Yes	"	No	No	Neo	E6971
pGL4.80[hRlucP/Neo]	Yes	"	hPEST	No	Neo	E6981
pGL4.81[hRlucCP/Neo]	Yes	"	hCL1-hPEST	No	Neo	E6991
pGL4.82[hRluc/Puro]	Yes	"	No	No	Puro	E7501
pGL4.83[hRlucP/Puro]	Yes	"	hPEST	No	Puro	E7511
pGL4.84[hRlucCP/Puro]	Yes	"	hCL1-hPEST	No	Puro	E7521

^Aluc2 = synthetic firefly luciferase gene. ^BhRluc = synthetic *Remilla* luciferase gene.

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[©]U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289 and 5,814,471, Australian Pat. No. 649289, European Pat. No. 0 553 234 and Japanese Pat. No. 3171595 have been issued to Promega Corporation for a beetle luciferase assay method, which affords greater light output with improved kinetics as compared to the conventional assay.

[©]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.

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