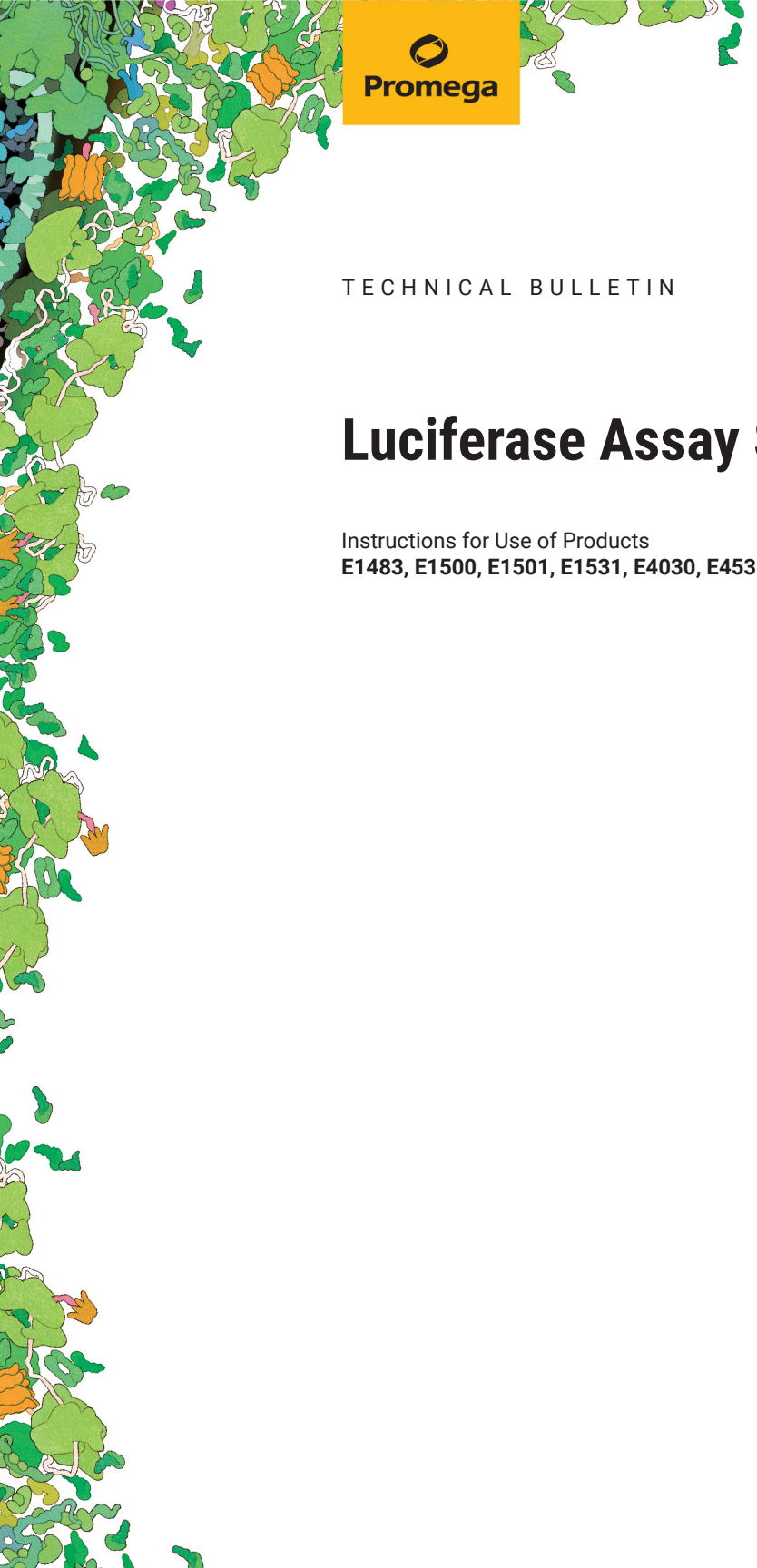

TECHNICAL BULLETIN

# Luciferase Assay System

Instructions for Use of Products
**E1483, E1500, E1501, E1531, E4030, E4530 and E4550.**

Revised 9/23
TB281

# 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 Bulletin.**
**E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com**

## 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 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•$Mg^{2+}$ 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.**

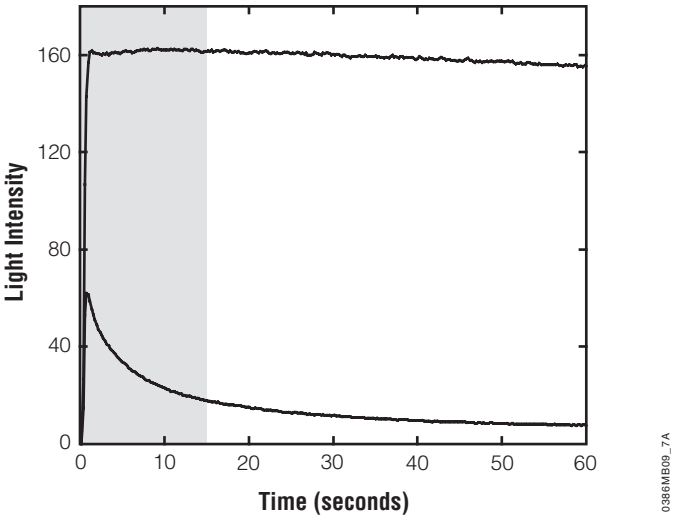


**Figure 2. Comparison of the Promega Luciferase Assay System to the conventional luciferase assay method.** NIH3T3 cells expressing the luciferase gene from Rous sarcoma virus were lysed with 1X Cell Culture Lysis Reagent 48 hours after infection. The shaded area represents the light typically lost 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.

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## 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 Conditions 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. Store Luciferase Assay Substrate and Luciferase Assay Buffer at –30°C to –10°C. Store Luciferase Assay Reagent at less than –65°C. Store reconstituted Luciferase Assay Reagent (Luciferase Assay Substrate + Luciferase Assay Buffer) in aliquots at –30°C to –10°C for up to 1 month or at less than –65°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 can be stored at room temperature and should be stored away from direct sunlight. Luciferase Cell Culture Lysis Reagent should be stored at –30°C to –10°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

There are 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 co-expression 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 |

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

[2]RLB 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 $K_2HPO_4$ (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 may 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:**

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

b. Cell lysates prepared using CCLR (Luciferase Cell Culture Lysis Reagent) will not yield optimal results when assaying for CAT, β-galactosidase or *Renilla* luciferase co-reporter 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® Discover and Explorer Systems are multimode detection instruments that measure Fluorescence Intensity, Luminescence and Absorbance while the GloMax® Discover is capable of measuring BRET and FRET. The GloMax® Systems can read 6-, 12-, 24-, 48-, 96- and 384-well plates and are configured with a factory-installed shaker for either linear or orbital shaking. The detection instruments have an optional heater, allowing precise temperature control from ambient temperature to 45°C. The integrated Tablet PC has touch screen interface. Label samples and see analyzed data and graphs from the instrument. Protocols for a variety of Promega assays are pre-installed.

#### 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.

### 5.B. Instrumentation (continued)

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 ($Mg^{2+}$- and $Ca^{2+}$-free)**

| | |
|---|---|
| 137mM | NaCl |
| 2.7mM | KCl |
| 4.3mM | $Na_2HPO_4$ |
| 1.4mM | $KH_2PO_4$ |

The final pH should be 7.4.

**lysozyme (5mg/ml)**

Add 1 volume of 1M $K_2HPO_4$ (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 new lysis mix for each use.

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## 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

| Product | Cat.# |
|---|---|
| GloMax® Discover System | GM3000 |
| GloMax® Explorer Fully Loaded Model | GM3500 |
| GloMax® Explorer with Luminescence and Fluorescence | GM3510 |

### 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 | *hRluc*[B] | 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 |

[A]*luc2* = synthetic firefly luciferase gene. [B]*hRluc* = synthetic *Renilla* luciferase gene.

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## 9. Summary of Changes

The following changes were made to the 9/23 revision of this document:

1. Expired patent statement was removed.
2 Expired luminometer information was removed.
3. Font and cover image were updated.