Luciferase Assay System

Promega

Technical Bulletin No. 281

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

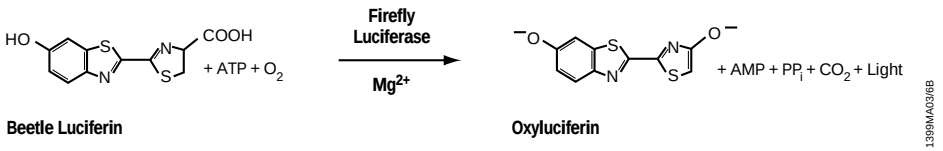

Figure 1. Bioluminescent reaction catalyzed by firefly luciferase.

Promega's Luciferase Assay System[a,b] 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. Promega's Luciferase Assay System incorporates coenzyme A (CoA) for improved kinetics (9), allowing greater enzymatic turnover 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).

Promega's 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 III.E); however, the Luciferase Assay System with Reporter Lysis Buffer (Cat.# E4030) is not suitable for these applications.

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 III). 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 III.B. The three lysis buffers are described in Section III.C and Table 1 recommends the appropriate lysis buffer for use with a particular cell type.

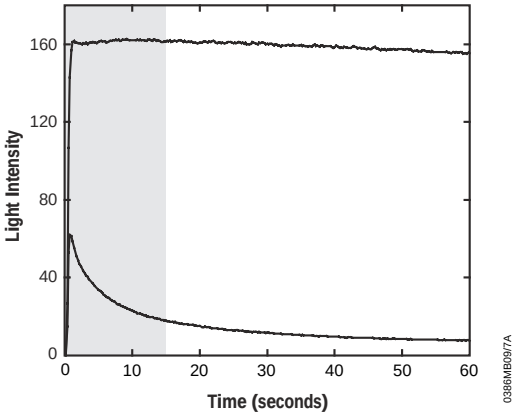

Figure 2. Comparison of Promega's Luciferase Assay System to the conventional luciferase assay method. NIH3T3 cells expressing the luciferase gene from Rous sarcoma virus were lysed with 1X Cell Culture Lysis Reagent 48 hours after infection. The shaded area represents the light typically lost in measurements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

II. Product Components

Product	Size	Cat.#
Luciferase Assay System[a,b]	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
- 1 Protocol

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

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

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

Product	Size	Cat.#
Luciferase Assay System with Reporter Lysis Buffer[a,b]	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
- 1 Protocol

Product	Size	Cat.#
Luciferase Assay System Freezer Pack[a,b]	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)
- 10 Protocols

Product	Size	Cat.#
Luciferase Reporter 1000 Assay System[a,b]	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
- 1 Protocol

Product	Size	Cat.#
Luciferase Assay Reagent[a,b]	1,000 assays	E1483

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

- 100ml Luciferase Assay Reagent
- 1 Protocol

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**. Luciferase Assay Reagent should be stored in aliquots and is stable at –20°C for up to 1 month, or at –70°C for up to 1 year after reconstitution or initial use. After preparation and freezing, the Luciferase Assay Reagent should be mixed well before use. Nonreconstituted system components may be stored at –20°C for 1 year. Store Luciferase Assay Substrate in the dark. Reporter Lysis Buffer may be stored at room temperature and should be stored away from direct sunlight. Cell Culture Lysis Reagent should be stored at –20°C.

Do not store the Luciferase Assay Reagent with dry ice.

Do not thaw the Luciferase Assay Reagent at temperatures above 25°C.

III. Preparations Prior to Performing the Luciferase Assay

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

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(c), Cat.# E1701).

B. Luciferase Assay Reagent Preparation

Note: Luciferase Assay Reagent is also available premixed (Cat.# E1483).

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**. 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.

C. Lysis Buffers

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

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.

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[a,b]	CCLR
plant cells[b]	CCLR
tissue homogenates	CCLR, RLB

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

[b]RLB has not been qualified for use with plant or bacterial cells.

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 VI), 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 to ensure complete lysis. **For 96-well plates, proceed to Section IV. 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 IV.

Note: An *Experienced User's Protocol* can be found at the end of this Technical Bulletin.

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 IV).
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 VI). Mix and incubate the cells for 10 minutes at room temperature. To assay the lysate, proceed to Section IV.
3. A protocol for the use of tissue homogenates with the Luciferase Assay System can be found in reference 10.

IV. Luciferase Assay Protocol

Material to Be Supplied by the User

- opaque multiwell plates or luminometer tubes

The following procedures (Section IV.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 III.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).

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

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.
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.

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.

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.

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.

V. General Considerations

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 IV) will result in a 5–10% decrease in enzyme activity.

Note: 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 autoluminescence (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.

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

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 III.A).

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.

C. Firefly Luciferase Reporter Vectors

The pGL3 Luciferase Reporter Vectors[c,d] contain the cDNA encoding luciferase (*luc*+) cloned from the North American firefly (*Photinus pyralis*) and a vector backbone that has been designed to provide enhanced reporter gene expression. Modifications that distinguish *luc*+ from the native luciferase gene (*luc*) fall into four general categories: i) the C-terminal tripeptide is substituted to eliminate peroxisome targeting of the expressed reporter enzyme; ii) codon usage is improved for increased expression in plant and animal cells; iii) two potential sites of N-glycosylation are modified; and iv) several DNA sequence changes are incorporated to disrupt extended palindromes, remove internal restriction sites and eliminate consensus sequences that may be recognized by genetic regulatory binding proteins. Changes in luciferase reporter activity directly correlate to the transcriptional activity of the cloned regulatory element when expressed in transfected cells. These modifications help to ensure that the luciferase reporter gene does not contribute spurious transcriptional signals. Further details on these modifications are provided in the *pGL3 Luciferase Reporter Vectors Technical Manual* #TM033.

In addition to changes made to the luciferase gene, four major modifications were incorporated into the vector backbone of the pGL3 family of luciferase vectors: i) the SV40 early poly(A) signal is replaced with the SV40 late poly(A) signal for improved RNA processing (13); ii) a synthetic poly(A) and transcription pause site is positioned upstream of the multiple cloning region to terminate spurious transcription, which may initiate within the vector backbone (14); iii) the small t intron is removed to eliminate cryptic splicing, resulting in greater reporter gene expression (15); and iv) the Kozak consensus sequence is added to increase the efficiency of luciferase translation initiation (16).

The modifications embodied in the pGL3 Vector family provide greater flexibility in performing genetic manipulations, minimal relative background activity and luciferase expression levels that are dramatically higher than previously obtained with the pGL2 Vectors[c]. Using the pGL3 Vectors, it is now possible to obtain measurable luciferase expression in cell types that are difficult to transfect, when studying weak promoter elements or when performing in vivo luminescence measurements. It is important to recognize that absolute light unit values and relative expression profiles of reporter vectors will vary between cell types. We recommend that the appropriate control vector always be included in experiments utilizing genetic reporter systems.

The pGL3 Vector family comprises four types of improved firefly luciferase vectors, the pGL3-Basic Vector, the pGL3-Promoter Vector, the pGL3-Enhancer Vector and the pGL3-Control Vector. The pGL3-Basic Vector (Cat.# E1751) lacks eukaryotic promoter and enhancer elements. The strategic placement of unique

restriction enzyme sites within this vector provides maximum flexibility in cloning and the ability to further manipulate putative genetic regulatory sequences. Expression of luciferase activity in cells transfected with the pGL3-Basic Vector is dependent upon the insertion of a functional promoter upstream of *luc*+. In addition, desired enhancer elements may be inserted at positions that flank the immediate promoter sequence or may be positioned downstream of *luc*+. The pGL3-Promoter Vector (Cat.# E1761) contains an SV40 promoter upstream of *luc*+. Genomic DNA fragments containing putative enhancer elements can be inserted in either orientation, upstream or downstream from the SV40 promoter/*luc*+ transcriptional unit. The pGL3-Enhancer Vector (Cat.# E1771) contains an SV40 enhancer downstream of the *luc*+ reporter gene. This allows verification of functional promoter/*luc*+ junctions when testing putative promoter sequences. The presence of an enhancer will, in many cases, provide increased transcriptional activity of cloned promoter elements. The pGL3-Control Vector (Cat.# E1741) contains both SV40 promoter and enhancer sequences, resulting in strong expression of luciferase activity in many types of eukaryotic cells. This plasmid is useful for general monitoring of transfection efficiency.

VI. 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 7.3.

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 fresh for each use.

VII. Related Products

Luciferase Assay Systems and Reagents

Product	Size	Cat.#
Reporter Lysis Buffer, 5X	30ml	E3971
Luciferase Cell Culture Lysis Reagent, 5X	30ml	E1531
Passive Lysis Buffer, 5X	30ml	E1941
Beetle Luciferin, Potassium Salt	5mg	E1601
	50mg	E1602
	250mg	E1603
QuantiLum® Recombinant Luciferase(c)	1mg	E1701
	5mg	E1702

Product	Size	Cat.#
Steady-Glo™ Luciferase Assay System(a,b)	10ml	E2510
	100ml	E2520
	10 × 100ml	E2550
Bright-Glo™ Luciferase Assay System(a,b)	10ml	E2610
	100ml	E2620
	10 × 100ml	E2650

Dual-Luciferase® Reporter Assay Systems

Product	Size	Cat.#
Dual-Luciferase® Reporter Assay System(a,b,e)	100 assays	E1910
Dual-Luciferase® Reporter Assay System 10-Pack(a,b,e)	1,000 assays	E1960
Dual-Luciferase® Reporter 1000 Assay System(a,b,e)	1,000 assays	E1980

Luciferase Reporter Vectors

Product	Size	Cat.#
pGL3-Control Vector (c,d)	20µg	E1741
pGL3-Enhancer Vector(c,d)	20µg	E1771
pGL3-Promoter Vector(c,d)	20µg	E1761
pGL3-Basic Vector(c,d)	20µg	E1751

Vectors are supplied with a glycerol stock of bacterial strain JM109.

Renilla Luciferase Control Reporter Vectors

Product	Size	Cat.#
pRL-SV40 Vector(f)	20µg	E2231
pRL-TK Vector(f)	20µg	E2241
pRL-CMV Vector(f,g)	20µg	E2261
pRL-null Vector(f)	20µg	E2271

Vectors are supplied with a glycerol stock of bacterial strain JM109. Please call Promega Technical Services or visit our Internet site at www.promega.com to inquire about bulk packaging and pricing information for individual pRL Vectors.

Luminometers (Single Sample)

Product	Cat.#
Turner Designs Luminometer Model TD-20/20 Genetic Reporter Instrumentation Package for Stabilized Assays	E2041
Turner Designs Luminometer Model TD-20/20 Genetic Reporter Instrumentation Package for Stabilized Assays with Printer	E2051
Turner Designs Luminometer Model TD-20/20 Genetic Reporter System with Single Auto Injector	E2351
Turner Designs Luminometer TD-20/20 Genetic Reporter System with Dual Auto Injector	E2361
Turner Designs Luminometer Model TD-20/20 Genetic Reporter Instrumentation Package with Printer, Auto Injector System	E2061

VIII. References

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Luciferase Assay System: *Experienced User's Protocol*

This quick protocol is intended as an easy-to-follow reminder for experienced users. Please follow the complete protocol (Sections III–IV) the first time you use the Luciferase Assay System.

Luciferase Assay Reagent Preparation (Section III.B)	1. Add Luciferase Assay Buffer to the vial containing the Luciferase Assay Substrate. Dispense into aliquots before freezing to avoid repeated freeze-thaw cycles.
Preparation of Mammalian Cell Lysates (Section III.D)	1. Remove growth media from cultured cells. 2. Rinse cells in 1X PBS. Do not dislodge cells. Remove as much of the PBS rinse as possible. 3. Dispense a minimal volume of 1X lysis buffer (CCLR, RLB or PLB) into each culture vessel. For culture dishes, scrape cells, vortex and centrifuge at 12,000 × *g* to pellet debris. Save supernatant. 4. Proceed to Luciferase Assay using standard conditions.
Preparation of Plant Tissue (Section III.E)	1. Quick-freeze the tissue in liquid nitrogen, grind the frozen tissue to a powder and resuspend in room temperature 1X CCLR with further homogenization. 2. Centrifuge to pellet debris. 3. Proceed to Luciferase Assay using standard conditions.
Preparation of Bacterial Cell Lysate (Section III.E)	1. Mix 40µl nontransformed bacteria (carrier cells) with 50µl of transformed culture. 2. Add 10µl of 1M K_2HPO_4 (pH 7.8) and 20mM EDTA. 3. Quick-freeze on dry ice, and then equilibrate to room temperature by placing the tube in room temperature water. 4. Add 300µl freshly prepared lysis mix (Section VI). Mix and incubate for 10 minutes at room temperature. 5. Proceed to Luciferase Assay using standard conditions.
Protocol for Manual Luminometers (Section IV.A)	1. Dispense 100µl of the Luciferase Assay Reagent into the appropriate number of luminometer tubes. 2. Program the luminometer to perform the appropriate delay and measurement times. Typically these are 2 and 10 seconds, respectively. 3. Add 20µl of cell lysate to the luminometer tube and mix by pipetting. 4. Initiate reading. The read times may be reduced if sufficient light is produced. Record the results.
Protocol for Single-Tube Luminometers with Injectors (Section IV.B)	1. Prime the luminometer injector with Luciferase Assay Reagent. 2. Dispense 20µl of cell lysate into a luminometer tube. 3. Program the luminometer to perform the appropriate delay and measurement times. Typically these are 2 and 10 seconds, respectively. 4. Place the tube in the luminometer and initiate the reading by injecting 100µl of Luciferase Assay Reagent. Record the results.
Protocol for Plate Reading Luminometers (Section IV.C)	1. Program the luminometer to perform the appropriate delay and measurement times. Typically these are 2 and 10 seconds, respectively. 2. Add 20µl of cell to plate wells. Place the plate on the luminometer. 3. Using the injector, add 100µl of Luciferase Assay Reagent to one plate well. 4. Measure and record the light produced. Repeat Steps 3 and 4 for each well.