Technical Manual

Renilla Luciferase Assay System

INSTRUCTIONS FOR USE OF PRODUCTS E2810 AND E2820.

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Promega

Renilla Luciferase Assay System

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1. Description

The *Renilla* Luciferase Assay System[a,b,c] was developed for reporter quantitation in mammalian cells. 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). *Renilla* luciferase, a monomeric 36kDa protein, catalyzes coelenterate-luciferin (coelenterazine) oxidation to produce light (3; Figure 1). Post-translational modification is not required for its activity, and the enzyme may function as a genetic reporter immediately following translation. The *Renilla* luciferase substrate, coelenterazine, also emits light from enzyme-independent oxidation, a process known as autoluminescence.

The *Renilla* Luciferase Assay System is designed to provide a fast and sensitive method of detecting sea pansy (*Renilla reniformis*) luciferase. This system is a convenient alternative to firefly (*Photinus pyralis*) reporter systems and is designed to yield reliable, linear results for a concentration range over seven orders of magnitude. The *Renilla* Luciferase Assay System has been formulated with a proprietary composition that significantly reduces the coelenterazine autoluminescence commonly seen with other reagents. The result is an assay system that is more sensitive than published methods by several orders of magnitude (3; Figure 2). This sensitivity is comparable to that seen with the firefly Luciferase Assay System. The *Renilla* Luciferase Assay System enables measurement of *Renilla* luciferase activity using either the wildtype or new synthetic *Renilla* luciferase as a primary reporter to study transcriptional regulation or as a co-reporter for normalization of experimental variations such as differences in transfection efficiencies. The *Renilla* Luciferase Assay System consists of *Renilla* Luciferase Assay Lysis Buffer, *Renilla* Luciferase Assay Buffer and *Renilla* Luciferase Assay Substrate. The *Renilla* Luciferase Assay Reagent and its preparation are described in Section IV.A.

For *Renilla* Luciferase Assay System citations visit: **www.promega.com/citations/**

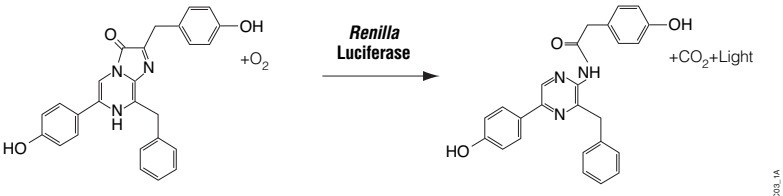

Figure 1. Bioluminescent reaction catalyzed by *Renilla* luciferase.

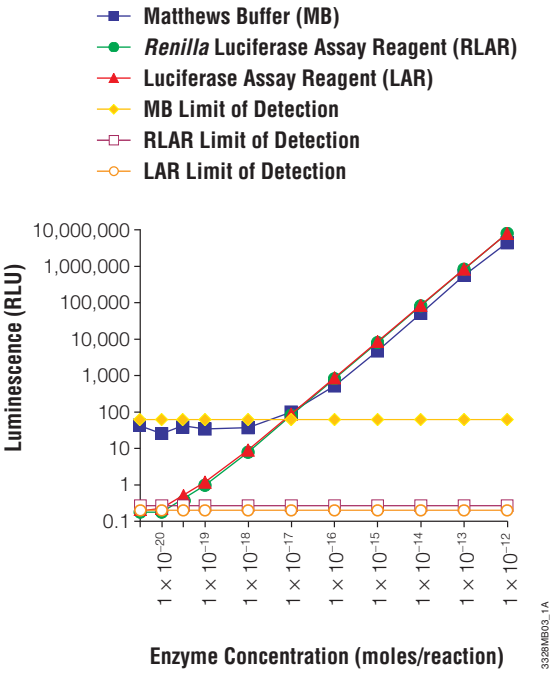

Figure 2. *Renilla* Luciferase Assay System has comparable sensitivity to the Luciferase Assay System (firefly luciferase) and 100-fold greater sensitivity than a published method for assaying *Renilla* luciferase. *Renilla* and firefly luciferase luminescence were compared in *Renilla* Luciferase Assay Reagent (RLAR) or Luciferase Assay Reagent (LAR), respectively, over a titration range of 1×10^{-12} to 3.56×10^{-20} moles/reaction. Twenty microliters of *Renilla* luciferase enzyme was diluted in *Renilla* Luciferase Assay Lysis Buffer or Matthews Lysis Buffer (3), and 20µl of firefly luciferase enzyme was diluted in Glo Lysis Buffer (Cat.# E2661). The diluted *Renilla* luciferase was then added to 100µl of RLAR or Matthews Buffer, while the diluted firefly luciferase was added to 100µl of LAR. Luminescence was measured on a Turner Designs Model 20e luminometer. Light emission was integrated over 10 seconds after an initial 2-second preread delay. Limit of detection values shown represent background plus two standard deviations and were determined for each assay by performing the assay without enzyme. Matthews Buffer composition is 0.5M NaCl, 0.1M potassium phosphate, 1.0mM Na_2EDTA, 1mg/ml porcine gelatin (pH 7.6). Matthews Lysis Buffer composition is 150mM HEPES (pH 8.0), 0.25% Triton® X-100, 1mg/ml porcine gelatin; 10% glycerol and 0.05% antifoam 289.

2. Product Components and Storage Conditions

Product	Size	Cat. #
Renilla Luciferase Assay System	100 assays	E2810

Each system contains sufficient reagents for 100 assays in 96-well plates. Includes:

- 100µl *Renilla* Luciferase Assay Substrate (100X)
- 10ml *Renilla* Luciferase Assay Buffer
- 30ml *Renilla* Luciferase Assay Lysis Buffer, 5X

Product	Size	Cat. #
Renilla Luciferase Assay System	1,000 assays	E2820

Each system contains sufficient reagents for 1,000 assays in 96-well plates. Includes:

- 1,000µl *Renilla* Luciferase Assay Substrate (100X)
- 100ml *Renilla* Luciferase Assay Buffer
- 30ml *Renilla* Luciferase Assay Lysis Buffer, 5X

Storage Conditions: Store the *Renilla* Luciferase Assay System at –20°C. It is best to prepare *Renilla* Luciferase Assay Reagent (Substrate + Buffer) before each use. If necessary, the prepared Reagent can be stored at –20°C for 2 weeks or at –70°C for up to 1 month.

Renilla Luciferase Assay Substrate contains a solvent that can be a mild irritant. Take appropriate precautions to prevent skin and eye contact. Careful pipetting of the substrate solvent is necessary because of the volatile nature of the solvent.

3. Preparation of Cell Lysates

Before performing the *Renilla* Luciferase Assay, prepare the *Renilla* Luciferase Assay Lysis Buffer as described in Section 3.A. Then prepare cell lysates following one of the procedures explained in Sections 3.B or 3.C.

Two procedures are described for the preparation of cell lysates using *Renilla* Luciferase Assay Lysis Buffer. The first procedure is recommended for passive lysis of cells cultured in multiwell plates (Section 3.B). The second procedure is intended for harvesting cells grown in culture dishes and for cell lysis by scraping of adherent cells (Section 3.C). The *Renilla* luciferase contained in the cell lysates prepared with *Renilla* Luciferase Assay Lysis Buffer is stable for 10 hours at room temperature (22°C) and for up to 24 hours at 4°C. (Stability is defined as ≤10% decrease in *Renilla* Luciferase Assay System activity.)

Freezing at –20°C is suitable for short-term storage of prepared lysates (up to 1 month). However, we recommend storing at –70°C for long-term storage. Subjecting cell lysates to more than 5 freeze-thaw cycles may result in gradual loss of *Renilla* luciferase enzyme activity.

Material to Be Supplied by the User
(Solution composition is provided in Section 5.A.)
- phosphate buffered saline (PBS)

3.A. Preparation of *Renilla* Luciferase Assay Lysis Buffer

Renilla Luciferase Assay Lysis Buffer is supplied as a 5X concentrate. Prepare a sufficient quantity of the 1X working solution by adding 1 volume of 5X *Renilla* Luciferase Lysis Buffer to 4 volumes of distilled water and mixing well. The diluted (1X) Lysis Buffer may be stored at 4°C for up to one month. However, we recommend that Lysis Buffer be prepared in the amount needed just before each experiment. Store the 5X *Renilla* Luciferase Assay Lysis Buffer at –20°C.

3.B. Lysis of Cells Cultured in Multiwell Plates

1. Determine transfection parameters (i.e., plated cell density and subsequent incubation time) to ensure that cells are no more than 95% confluent at the desired time of lysate preparation. Remove the growth medium from the cultured cells, and gently add a sufficient volume of phosphate buffered saline (PBS) to wash the surface of the culture vessel. Swirl the vessel briefly to remove detached cells and residual growth medium. Completely remove the rinse solution before adding *Renilla* Luciferase Assay Lysis Buffer.

Use only *Renilla* Luciferase Assay Lysis Buffer with the *Renilla* Luciferase Assay System. Other lysis buffers may inhibit the assay.

2. To each culture well add the minimum volume of 1X *Renilla* Luciferase Assay Lysis Buffer required to completely cover the cell monolayer. The recommended volumes of *Renilla* Luciferase Assay Lysis Buffer to be added per well are shown in Table 1.

Table 1. Recommended Volumes of 1X *Renilla* Luciferase Assay Lysis Buffer for Use in Multiwell Plates.

Multiwell Plate Size	1X Lysis Buffer Per Well
6-well culture plate	500µl
12-well culture plate	250µl
24-well culture plate	100µl
48-well culture plate	65µl
96-well culture plate	20µl

3.B. Lysis of Cells Cultured in Multiwell Plates (continued)

3. Place the culture plates on a rocking platform or orbital shaker with gentle rocking/shaking to ensure complete and even coverage of the cell monolayer with 1X *Renilla* Luciferase Assay Lysis Buffer. Rock the culture plates at room temperature for 15 minutes.

4. Transfer the lysate to a tube or vial for further handling and storage. Alternatively, reporter assays may be performed directly in the wells of the culture plate. In general, it is unnecessary to clear lysates of residual cell debris prior to performing the *Renilla* Luciferase Assay. However, if subsequent protein determinations are to be performed, we recommend clearing the lysate samples by centrifugation for 30 seconds at top speed in a refrigerated microcentrifuge. Alternatively a centrifuge with plate adapters can be used. Transfer cleared lysates to a fresh tube prior to reporter enzyme analyses.

Notes:

1. Cultures that are overgrown are often more resistant to complete lysis and typically require an increased volume of *Renilla* Luciferase Assay Lysis Buffer and/or an extended treatment period to ensure complete lysis. *Renilla* luciferase is stable in cell lysates prepared with *Renilla* Luciferase Assay Lysis Buffer; therefore, extending the period of lysis treatment will not compromise the activity of *Renilla* luciferase.

2. Microscopic inspection of different cell types treated for lysis may reveal seemingly different lysis results. Treatment of many types of cultured cells with *Renilla* Luciferase Assay Lysis Buffer produces complete dissolution of their structure within a 15-minute period. However, *Renilla* Luciferase Assay Lysis Buffer treatment of some cell types may result in discernible cell silhouettes on the surface of the culture well or large accumulations of floating debris. Despite the appearance of such cell remnants, we typically find complete solubilization of *Renilla* luciferase within a 15-minute treatment period. Nevertheless, some types of cultured cells may exhibit greater inherent resistance to lysis, and optimizing the treatment conditions may be required.

3.C. Lysis of Cells by Scraping

1. Remove growth medium from the cultured cells and gently apply a sufficient volume of PBS to rinse the cells on the bottom of the culture vessel. Swirl the vessel briefly to remove detached cells and residual growth medium. Take care to completely remove the rinse solution before adding the 1X *Renilla* Luciferase Assay Lysis Buffer.

Use only *Renilla* Luciferase Assay Lysis Buffer with the *Renilla* Luciferase Assay System. Other lysis buffers may inhibit the assay.

2. Homogenous lysates may be prepared rapidly by manually scraping the cells from culture dishes in the presence of 1X *Renilla* Luciferase Assay Lysis Buffer. The recommended volumes of *Renilla* Luciferase Assay Lysis Buffer to be added per culture dish are listed in Table 2.

Table 2. Recommended Volumes of *Renilla* Luciferase Assay Lysis Buffer for Use with the Cell Scraping Protocol.

Cell Culture Plate Size	1X Lysis Buffer Per Well
100 × 20mm	1ml
60 × 15mm	400µl
35 × 12mm	200µl
6-well culture plate	250µl
12-well culture plate	100µl

Certain cell types may exhibit greater inherent resistance to lysis, and optimizing the treatment conditions may be required.

3. Cells may be harvested immediately following the addition of *Renilla* Luciferase Assay Lysis Buffer by scraping with a disposable plastic cell lifter or a rubber policeman. Tilt the plate, and scrape the lysate down to the lower edge. Pipet the accumulated lysate several times to obtain a homogenous suspension. If the scraper is used to prepare more than one sample, thoroughly clean the scraper between uses.

4. Transfer the lysate into a tube or vial for further handling and storage. The cell lysate can undergo 1 or 2 freeze-thaw cycles to ensure complete lysis of cells. Generally, it is unnecessary to clear lysates of residual cell debris prior to performing the *Renilla* Luciferase Assay. However, if subsequent protein determinations are to be performed, we recommend clearing the lysate samples by centrifugation for 30 seconds in a refrigerated microcentrifuge.

5. Transfer the cleared lysates to a fresh tube prior to reporter enzyme analyses.

4. *Renilla* Luciferase Assay Protocol

4.A. Preparation of *Renilla* Luciferase Assay Reagent

Materials to Be Supplied by the User

- luminometer
- siliconized polypropylene tube or small glass vial

1. *Renilla* Luciferase Assay Reagent (Substrate + Buffer) is best when prepared before each use. It is stable for 12 hours at room temperature. If necessary, *Renilla* Luciferase Assay Reagent may be stored at –20°C for 2 weeks or at –70°C for up to 1 month. It may be thawed at room temperature up to five times without appreciable activity loss. **Note:** Stability is defined as ≤10% decrease in *Renilla* Luciferase Assay System activity.

Renilla Luciferase Assay Substrate contains a solvent that can be a mild irritant. Appropriate precautions should be taken to prevent skin and eye contact. Careful pipetting of the solvent is necessary because of the solvent's volatile nature.

2. Prepare an adequate volume to perform the desired number of *Renilla* Luciferase Assays (100µl of reagent per assay). Add 1 volume of 100X *Renilla* Luciferase Substrate to 100 volumes of *Renilla* Luciferase Assay Buffer in a glass or siliconized polypropylene tube. If the entire volume of *Renilla* Luciferase Assay Buffer is to be made into *Renilla* Luciferase Assay Reagent, the *Renilla* Luciferase Assay Buffer bottle may be used.

   Example 1 (10 assays)

   Add 10µl of 100X *Renilla* Luciferase Assay Substrate to 1ml of *Renilla* Luciferase Assay Buffer contained in either a glass vial or siliconized polypropylene tube. This will prepare sufficient *Renilla* Luciferase Assay Reagent to perform 10 assays.

   Example 2 (100 assays)

   Transfer 10ml of *Renilla* Luciferase Assay Buffer into a glass vial or siliconized polypropylene tube or measure 10ml of *Renilla* Luciferase Assay Buffer in a 10ml pipette, discard the residual buffer in the bottle and return the 10ml of *Renilla* Luciferase Assay Buffer to the bottle. Add 100µl of 100X *Renilla* Luciferase Assay Substrate (RLAS) to the 10ml of *Renilla* Luciferase Assay Buffer. Rinse the pipette tip used to transfer the RLAS in the newly prepared Reagent to wash any residual substrate from the pipette tip.

4.B. Standard Protocol

The following protocol is designed for use with manual luminometers or other luminometers fitted with one reagent injector.

Prior to beginning this protocol, verify that the *Renilla* Luciferase Assay Reagent has been prepared recently or has been thawed in a room temperature water bath and mixed prior to use, as thawing generates density and composition gradients.

If using a manual luminometer:

1. Add 100µl of *Renilla* Luciferase Assay Reagent to the luminometer tube.
2. Add 20µl of cell lysate. Mix quickly by flicking the tube with a finger or vortex to thoroughly mix (1–2 seconds).
3. Place tube in luminometer and initiate measurement. Luminescence is normally integrated over 10 seconds with a 2-second delay. Other integration times may also be used.
4. If the luminometer is not connected to a printer or computer, record the *Renilla* luciferase activity measurement.
5. Discard the reaction tube, and proceed to the next *Renilla* Luciferase Assay reaction, repeating Steps 1–4.

If using a luminometer fitted with one reagent injector:

1. Format the luminometer so that the injector dispenses 100µl. Prime the injector with *Renilla* Luciferase Assay Reagent.

Priming Assay Reagent through an empty injector system prevents dilution and contamination of the primed reagent.

Note: To prime auto-injector systems with little or no loss of *Renilla* Luciferase Assay Reagent we recommend first purging all storage liquid (i.e., deionized water or ethanol wash solution; Section IV.C) from the injector system. Priming assay reagent through an empty injector system prevents dilution and contamination of the primed reagent. Thus, the volume of primed reagent may be recovered and returned to the reservoir of bulk reagent.

2. For each reaction, carefully add 20µl of cell lysate to an individual luminometer tube or to the wells of a multiwell plate.
3. Place the samples in a luminometer.
4. Initiate measurement. This action will cause *Renilla* Luciferase Assay Reagent to be injected into the reaction vessel and the measurement to be taken subsequently. Luminescence is normally integrated over 10 seconds with a 2-second delay. Other integration times may also be used.
5. If the luminometer is not connected to a printer or computer, record the *Renilla* luciferase activity measurement.

If using a luminometer fitted with one reagent injector (continued):

6. If using a single-tube luminometer, discard the reaction tube, and proceed to the next *Renilla* Luciferase Assay reaction. If using a plate luminometer, the luminometer will automatically begin injecting *Renilla* Luciferase Assay Reagent into the next well indicated on the luminometer plate.

4.C. Cleaning Reagent Injectors

1. Purge *Renilla* Luciferase Assay Reagent from the injector lines by repeated priming/washing with a volume of deionized water equivalent to 3 pump void volumes.
2. Prepare 70% ethanol in water as wash reagent. Prime the system with at least 5ml of 70% ethanol to completely replace the void volume and rinse the injector plumbing. It is preferable to allow the injector to soak in this wash solution for 30 minutes prior to rinsing with deionized water.
3. Rinse with a volume of deionized water equivalent to at least 3 pump void volumes to thoroughly remove all traces of ethanol.

4.D. Wash Protocol for the Injectors in the GloMax™ 20/20 Luminometers

1. Rinse the *Renilla* Luciferase Assay Reagent from the injector by performing 10 priming cycles with deionized water.
2. Perform at least 10 priming cycles with 70% ethanol, and allow tubing to soak in this wash solution for 30 minutes.
3. Perform at least 10 priming cycles with deionized water to remove all traces of ethanol.

4.E. Determining Assay Backgrounds

The expression of a luciferase reporter is quantitated as the luminescence produced above background levels. In most cases, because the background created by the reagent in the absence of *Renilla* luciferase is exceptionally low, this luciferase activity is directly proportional to total luminescence. However, when measuring very small amounts of luciferase it is important to subtract the background signal from the measurement of total luminescence. Background luminescence in the measurement of *Renilla* luciferase activity can arise from two possible sources:

- Instrument and sample tube background luminescence.
- Autoluminescence of the *Renilla* luciferase substrate, coelenterazine. This is caused by nonenzymatic oxidation of the coelenterazine in solution.

The background luminescence contributed by the sources noted above is consistent throughout the experiment and can be subtracted from subsequent measurements of *Renilla* luciferase. To determine the background contributed by the instrument, the sample tube and coelenterazine autoluminescence, perform the following procedure:

Five to ten background readings should be performed and the mean reading used to obtain a statistically significant value for instrument and sample tube background.

1. Use *Renilla* Luciferase Assay Lysis Buffer to prepare a lysate of nontransfected control (NTC) cells.
2. Perform the *Renilla* Luciferase Assay as for experimental samples (Section 4.B).
3. Measure the luminescence (this is the background luminescence).
4. Subtract the background luminescence as determined in Step 3 from the experimental sample luminescence.

5. Appendix

5.A. Composition of Buffers and Solutions

phosphate buffered saline (PBS, 10X)

11.5g	Na_2HPO_4
2g	KH_2PO_4
80g	NaCl
2g	KCl

Dissolve in 1 liter of sterile, deionized water. The pH of 1X PBS will be 7.4.

5.B. Description of the phRL Family of Synthetic *Renilla* Luciferase Reporter Vectors

The phRL family of *Renilla* control vectors consists of seven vectors in which a synthetic *Renilla* luciferase gene sequence has been inserted for more efficient mammalian expression and reduced risk of anomalous transcriptional behavior. The *Renilla* gene has been redesigned by a systematic approach in which codons have been changed to those most frequently used in mammals, while simultaneously removing most of the consensus sequences of the transcription factor binding sites. The resulting DNA sequence changed the second amino acid of the protein from Thr in the wildtype to Ala, introducing a Kozak sequence that may result in better expression efficiency. The potential transcription factor binding sites are reduced from around 300 in the native gene to only a few in the synthetic *Renilla* luciferase gene. This synthetic *Renilla* luciferase gene is cloned into a variety of expression vector backbones to provide a series of vectors to fit various experimental conditions.

5.B. Description of the phRL Family of Synthetic *Renilla* Luciferase Reporter Vectors (continued)

The phRL family of *Renilla* control vectors provide significant advantages over the prior pRL *Renilla* control vectors:

- **Improved expression levels:** The synthetic *Renilla* luciferase gene supports a significant increase in *Renilla* luciferase expression in mammalian cells. Depending on the vector and cell line used, up to a 300-fold increase in expression may be obtained.
- **Improved reliability of the control reporter:** The synthetic *Renilla* luciferase gene exhibits reduced risk of anomalous transcriptional behavior, and the co-reporter expression is less likely to be influenced by experimental treatments.
- **Different promoter and vector backbones:** A variety of configurations are available to support both adequate expression and reliability of the control reporter under different experimental conditions.
- **Vectors are purified** to the extent that they may be used directly in transfection without the need for further manipulation.

5.C. Impact of the Synthetic Vectors with *Renilla* Luciferase Assay Reagent

Depending on the vector backbone and the cell line used in an experiment, up to a 300-fold increase in activity can be detected when using the synthetic *Renilla* gene compared to results determined with the native gene found in the previous generation of pRL Vectors. Sensitivity is further enhanced by the improvements found in *Renilla* Luciferase Assay System compared to other methods (i.e., Matthews Buffer; Figure 3). The *Renilla* Luciferase Assay Reagent combined with the phRL synthetic vectors creates opportunities for the most robust, highly sensitive reporter assay system available.

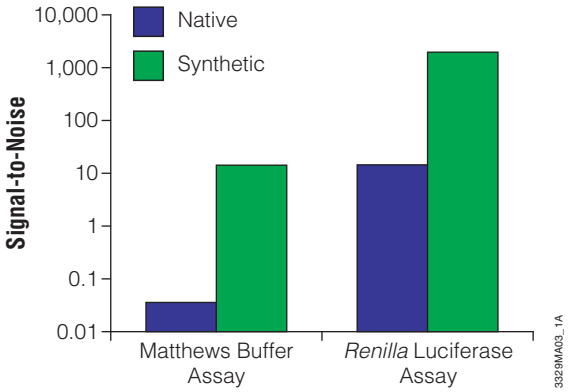

Figure 3. The *Renilla* Luciferase Assay System was used to measure expression from the early generation of Promega *Renilla* vectors containing the native *Renilla* gene and from the phRL Vectors containing the synthetic *Renilla* gene. *Renilla* luciferase, expressed in CHO cells transfected with either the pRL-TK(Int$^-$) (native gene) or the phRL-TK(Int$^-$) (synthetic gene), was measured using either *Renilla* Luciferase Assay Reagent or Matthews Buffer (3; compositions as noted in Figure 2) as follows. CHO cells containing *Renilla* luciferase were lysed in *Renilla* Luciferase Assay Lysis Buffer or Matthews Buffer. Twenty-microliter aliquots of lysate containing the native or synthetic gene were added to 100µl of *Renilla* Luciferase Assay Reagent or Matthews Buffer. Luminescence was measured on a Turner Designs Model 20e luminometer. Light emission was integrated over a 10-second read after an initial 2-second preread delay. Transfection efficiencies for each set of vectors were normalized to firefly enzyme, which was co-transfected in the same cells. Firefly luciferase was measured with Luciferase Assay Reagent (LAR).

5.D. Instrument Considerations

Single-Sample Luminometers

The *Renilla* Luciferase Assay Reagent exhibits reaction kinetics in which the signal half-life is at least 2 minutes (Figure 4). Thus, single-sample luminometers designed for low-throughput applications do not require reagent injectors to perform *Renilla* luciferase assays. Luminometers should be configured to measure light emission over a defined period, rather than measuring "flash" intensity or "peak" height. For the standard *Renilla* Luciferase Assay, we recommend programming luminometers to provide a 2-second preread delay, followed by a 10-second measurement period. However, depending on the type of instrument and the number of samples being processed, some users may prefer to shorten the period of premeasurement delay and/or the period of luminescence measurement.

5.D. Instrument Considerations (continued)

For convenience, one can equip the luminometers with a computer or an online printer for direct capture of data output, eliminating the need to pause between samples to manually record the measured values.

The GloMax™ 20/20 Luminometer (Cat.# E5311) and GloMax™ 20/20 Luminometer equipped with single or dual auto-injector (Cat.# E5321 or E5331) and printer are ideally suited for low-throughput processing of *Renilla* luciferase assays. The GloMax™ 20/20 Luminometer is preprogrammed to perform injections and readings of *Renilla* luciferase reporter activity with a single "Start" command. Furthermore, the instrument is programmed to provide individual luminescence measurements as well as statistical analyses of values measured within replicate groups.

Multiple-Sample and Plate-Reading Luminometers

The most convenient method of performing large numbers of *Renilla* Luciferase Assay measurements is to use a luminometer capable of processing multiple sample tubes or by configuring assays in a 96-well plate and using a plate-reading luminometer.

For high-throughput applications, we recommend first dispensing the desired volume of each sample into the individual assay tubes or wells of the microplate. *Renilla* luciferase assays are then performed according to the following steps: (i) inject *Renilla* Luciferase Assay Reagent, and (ii) measure luminescence from *Renilla* luciferase. Therefore, multisample and plate-reading luminometers should be equipped with at least one reagent injector to perform the *Renilla* Luciferase Assay.

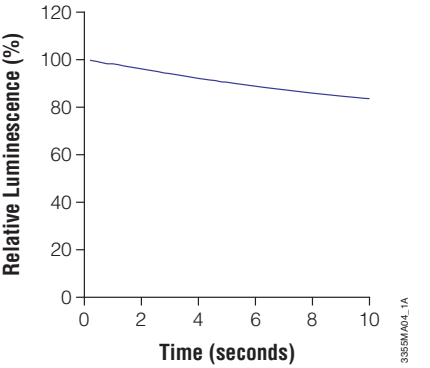

Figure 4. Signal profile collected over ten seconds shows signal stability. Purified *Renilla* enzyme was diluted in *Renilla* Luciferase Assay Reagent. A 20µl aliquot of enzyme was mixed with 100µl of *Renilla* Luciferase Assay Reagent, and *Renilla* luciferase activity was measured immediately. Although data was collected using a continuous-read luminometer (the Orion Berthold Detection System, a 96-well plate luminometer), similar data can be achieved using the GloMax™ 20/20 Luminometer with a 2-second pre-integration delay.

Note: It is common for the luminescence intensity of luciferase-mediated reactions to exceed the linear range of a luminometer. Verify that your luminometer provides a diagnostic warning when the luminescence of a given sample exceeds the linear range of the photomultiplier tube. If it does not, it is important to establish the luminometer linear range of detection prior to performing *Renilla* luciferase reporter assays. Purified *Renilla* luciferase or *Renilla* luciferase expressed in cell lysates may be used to determine the working range of a particular luminometer. Perform serial dilutions of purified *Renilla* luciferase in 1X *Renilla* Luciferase Assay Lysis Buffer containing 1mg/ml porcine gelatin. Gelatin may be omitted if cell lysates are used. The addition of exogenous protein can help to ensure stability of the purified *Renilla* luciferase enzyme at extremely dilute concentrations. However, all purified enzyme dilutions should be used within three hours of their preparation to ensure optimal performance.

5.E. References

1. Wood, K.V. *et al.* (1984) Synthesis of active firefly luciferase by in vitro translation of RNA obtained from adult lanterns. *Biochem. Biophys. Res. Comm.* **124**, 592–6.
2. de Wet, J.R. *et al.* (1985) Cloning of firefly luciferase cDNA and the expression of active luciferase in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **82**, 7870–3.
3. Matthews, J.C., Hori, K. and Cormier, M.J. (1977) Purification and properties of *Renilla reniformis* luciferase. *Biochemistry* **16**, 85–91.

5.F. Related Products

pGL4 Synthetic *Renilla* (*hRluc*) Luciferase Reporter Vectors

Product	Multiple Cloning Region	Protein Degradation Sequence	Reporter Gene Promoter	Mammalian Selectable Marker	Cat.#
pGL4.70[*hRluc*]	Yes	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

5.F. Related Products (continued)

Luciferase Reporter Vector Sequencing Primers

Product	Size	Cat.#
RVprimer3 (clockwise)	2µg	E4481
RVprimer4 (counterclockwise)	2µg	E4491

Luciferase Assay Systems

Product	Size	Cat.#
Dual-Glo™ Luciferase Assay System	10ml	E2920
	100ml	E2940
	10 × 100ml	E2980
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™ 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 Systems

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

[a]U.S. Pat. Nos. 7,078,181, 7,108,996 and 7,118,878, Australian Pat. No. 2001275325 and other patents pending.

[b]This product does not convey a license to use recombinant Renilla luciferase under U.S. Pat. Nos. 5,292,658, 5,418,155 and related patents. Promega sells licensed Renilla luciferase vectors, which may be used in conjunction with this product.

[c]Certain applications of this product may require licenses from others.