Technical Manual

Renilla-Glo™ Luciferase Assay System

INSTRUCTIONS FOR USE OF PRODUCTS E2710, E2720, AND E2750.

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Renilla-Glo™ Luciferase Assay System

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1. Description

Bioluminescence is the pre-eminent detection method for performing genetic reporter assays. The traditional reporter of choice has been firefly luciferase, but recently the need for reporters that are smaller and do not require ATP has resulted in the increased use of other luciferases, such as the copepod luciferases. The best studied of this class is *Renilla* luciferase, the bioluminescent enzyme derived from the sea pansy, *Renilla reniformis* (Figure 1). The enzyme is about half the size of firefly (36kDa rather than 61kDa), does not require ATP, and is already featured in some Promega reporter gene assays. In a dual-enzyme format, firefly and *Renilla* luciferases can be used as co-reporters in both high-sensitivity (Dual-Luciferase® Reporter Assay) or high-signal stability (Dual-Glo® Luciferase Assay) systems. In a single-enzyme format, the *Renilla* Luciferase Assay is highly sensitive, but previously a high-signal stability assay was not available.

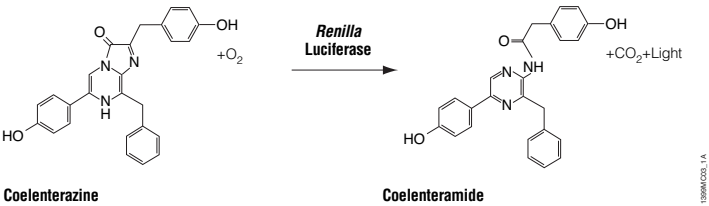

Figure 1. Bioluminescent reaction catalyzed by *Renilla* luciferase.

The *Renilla*-Glo™ Luciferase Assay System[a–c] is a single-addition reagent that generates a glow-type signal with *Renilla* luciferase. When reconstituted, it has the capacity to lyse cells, reduce the autoluminescence of the coelenterazine substrate, and produce a stable signal (i.e., half-life greater than 60 minutes at 22°C).

2. Product Components and Storage Conditions

Product	Size	Cat.#
Renilla-Glo™ Luciferase Assay System	10ml	E2710

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

- 100µl *Renilla*-Glo™ Luciferase Assay Substrate
- 10ml *Renilla*-Glo™ Luciferase Assay Buffer

Product	Size	Cat.#
Renilla-Glo™ Luciferase Assay System	100ml	E2720

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

- 1ml *Renilla*-Glo™ Luciferase Assay Substrate
- 100ml *Renilla*-Glo™ Luciferase Assay Buffer

Product	Size	Cat.#
Renilla-Glo™ Luciferase Assay System	10 × 100ml	E2750

Each system contains sufficient reagent for 10,000 assays in 96-well plates. Includes:

- 10 × 1ml *Renilla*-Glo™ Luciferase Assay Substrate
- 10 × 100ml *Renilla*-Glo™ Luciferase Assay Buffer

Storage Conditions: Store the *Renilla*-Glo™ Luciferase Assay System at –20°C. Do not thaw above 25°C. The buffer may be stored at 4°C for 1 year or at room temperature for 3 months, and it can be frozen and thawed at least 10 times without any change in performance. The substrate may be stored at 4°C for up to two weeks. Once reconstituted, the reagent will lose 10% activity in ~2 hours and 50% activity in ~12 hours at room temperature. The stability of the reconstituted reagent is greater at 4°C (10% loss in ~10 hours), but we recommend preparing the reagent immediately before use and not storing reconstituted reagent at any temperature.

3. *Renilla*-Glo™ Luciferase Assay Protocol

3.A. Preparation of the Reconstituted Reagent

Add one volume of 100X *Renilla*-Glo™ Luciferase Assay Substrate to 100 volumes of *Renilla*-Glo™ Luciferase Assay Buffer in order to generate an amount of *Renilla*-Glo™ Luciferase Assay Reagent sufficient to perform the desired experiment. For example, if the experiment requires 10ml of reagent, add 100µl of substrate to 10ml of buffer.

3.B. Standard Protocol

1. Allow all assay components (reagent and sample) to equilibrate to room temperature prior to assay.
2. Add one volume of *Renilla*-Glo™ Luciferase Assay Reagent equal to the volume of the sample and mix.
3. Wait at least 10 minutes, and measure the luminescence. Signals generated before 10 minutes do not decay with a glow-type signal (see Section 5, Appendix).

   Note: While the *Renilla*™-Glo Luciferase Assay is designed to be used in an add-and-read format, it is possible to use a lysate as a sample. We recommend making the lysates using Glo Lysis Buffer (Cat.# E2661) or Passive Lysis Buffer (Cat.# E1941) for best results.

4. Related Products

Product	Size	Cat.#
Renilla Luciferase Assay System	100 assays	E2810
Dual-Luciferase® Reporter Assay System	100 assays	E1910
Dual-Glo® Luciferase Assay System	10ml	E2920
Bright-Glo™ Luciferase Assay System**	10ml	E2610
Steady-Glo® Luciferase Assay System	10ml	E2510
ONE-Glo™ Luciferase Assay System**	10ml	E6110
Glo Lysis Buffer	100ml	E2661
Passive Lysis Buffer	30ml	E1941
EnduRen™ Live Cell Substrate	0.34mg	E6481
ViviRen™ Live Cell Substrate	0.37mg	E6491
GloMax® Multi+ Detection System	each	E8031

*For Laboratory Use.
Additional sizes available.

4. Related Products (continued)

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

Product Cat.#	Multiple Cloning Region	Protein Degradation Sequence	Reporter Gene Promoter	Mammalian Selectable Marker
pGL4.70[*hRluc*] E6881	Yes	No	No	No
pGL4.71[*hRlucP*] E6891	Yes	hPEST	No	No
pGL4.72[*hRlucCP*] E6901	Yes	hCL1-hPEST	No	No
pGL4.73[*hRluc*/SV40] E6911	No	No	SV40	No
pGL4.74[*hRluc*/TK] E6921	No	No	HSV-TK	No
pGL4.75[*hRluc*/CMV] E6931	No	No	CMV	No
pGL4.76[*hRluc*/Hygro] E6941	Yes	No	No	Hygro
pGL4.77[*hRlucP*/Hygro] E6951	Yes	hPEST	No	Hygro
pGL4.78[*hRlucCP*/Hygro] E6961	Yes	hCL1-hPEST	No	Hygro
pGL4.79[*hRluc*/Neo] E6971	Yes	No	No	Neo
pGL4.80[*hRlucP*/Neo] E6981	Yes	hPEST	No	Neo
pGL4.81[*hRlucCP*/Neo] E6991	Yes	hCL1-hPEST	No	Neo
pGL4.82[*hRluc*/Puro] E7501	Yes	No	No	Puro
pGL4.83[*hRlucP*/Puro] E7511	Yes	hPEST	No	Puro
pGL4.84[*hRlucCP*/Puro] E7521	Yes	hCL1-hPEST	No	Puro

5. Appendix

5.A. Cells vs. Purified Luciferase

The *Renilla*-Glo™ Luciferase Assay System was characterized using both *Renilla* luciferase expressed in transfected cells and purified *Renilla* luciferase. There is no difference in performance between the expressed and purified enzymes, as shown Figure 2.

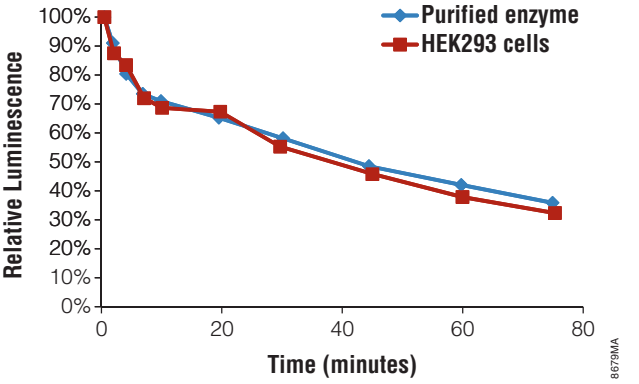

Figure 2. Reaction kinetics for both *Renilla* luciferase expressed in transfected cells and purified *Renilla* luciferase. Expressed enzyme was created by plating HEK293 cells at ~10,000 cells/well and transfecting them with pGL4.74 [*hRluc*/TK]. The purified enzyme is at a concentration of 70ng/ml. Both cells and purified enzyme were assayed in DMEM without phenol red + 0.1% Prionex® in a volume of 50µl.

5.B. Kinetics of the *Renilla* Luciferase Reaction

The reaction between *Renilla* luciferase and native coelenterazine produces a luminescent signal that decays in two phases (Figure 3). The first phase accounts for 65% of the signal amplitude but decays rapidly with a half-life of ~2.5 minutes. The second phase is not as bright (35% of the signal) but is much more stable, with a half-life of ~70 minutes. The reaction between *Renilla* luciferase and coelenterazine-h is almost identical to the native reaction, but the amplitude of the first phase is much lower (Figure 3). Thus, in a flash-type reaction, the greater overall brightness of native coelenterazine makes it the preferred substrate. However, in a glow-type reaction, it is advantageous to minimize the impact of any rapidly decaying signals. Hence, to enhance the stability of the signal produced in the *Renilla*-Glo™ Luciferase Assay: 1) coelenterazine-h has been chosen as the substrate, and 2) it is recommended to wait 10 minutes between addition of reagent and measurement of the luminescent signal. **Note**: These reaction kinetics are specific to *Renilla* luciferase. While luciferases from other species may use a coelenterazine substrate, not all luciferases are reported to catalyze coelenerazine-h with the same efficiency as *Renilla* luciferase.

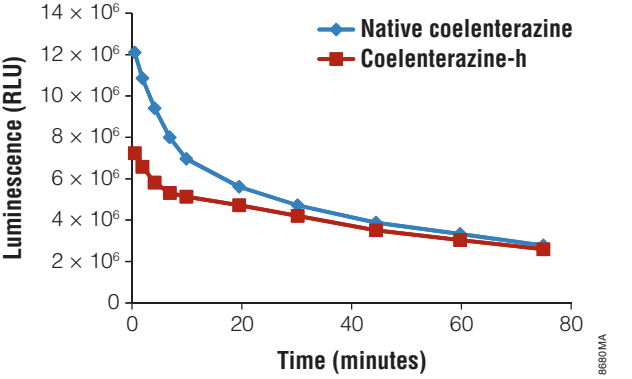

Figure 3. Reaction kinetics of *Renilla* luciferase with different substrates. Native coelenterazine (30µM) or coelenterazine-h (22µM) was added to the *Renilla*-Glo™ Luciferase Assay Buffer, a volume of 50µl was added to 50µl of purified enzyme (70ng/ml in DMEM without phenol red + 0.1% Prionex®), and the luminescent signal was monitored over time.

5.C. Conditions Affecting Assay Performance

The *Renilla*-Glo™ Luciferase Assay System was characterized in a variety of media (Figure 4). In most cases, the luminescence was ± 20% of that observed with DMEM without phenol red; the notable exceptions included media containing phenol red (e.g., DMEM and MEMα), which is known to absorb the light emitted from luciferases and hence quench the signal observed. In all media tested, the half-life was greater than 60 minutes.

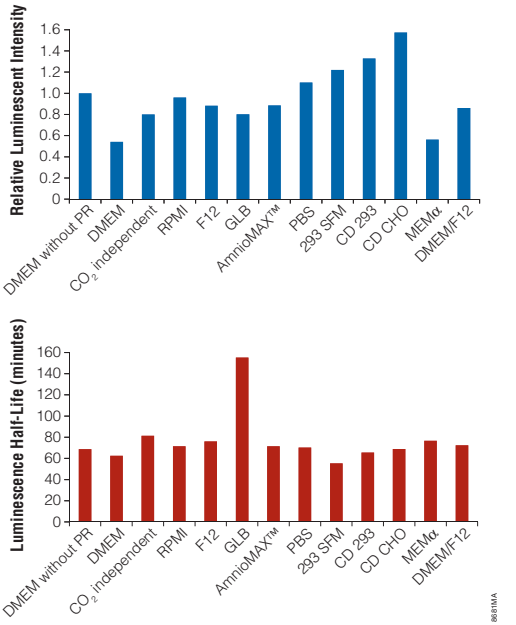

Figure 4. Relative luminescence and signal stability of *Renilla* luciferase in a variety of media. The *Renilla*-Glo™ Luciferase Assay Reagent was mixed (50µl + 50µl) with 70ng/ml of recombinant *Renilla* luciferase in a variety of media. The reaction was monitored as in Figure 3. The relative intensity was determined from the signal measured 10 minutes after reagent addition and normalized to DMEM without phenol red. The half-life was calculated from the decay of the signal from 10 to 75 minutes. GLB = Glo Lysis Buffer (Cat.# E2661).

5.C. Conditions Affecting Assay Performance (continued)

Aside from the choice of media, there are other common components in standard reporter assays, such as sera and solvents, that could have an impact on the signal produced by *Renilla* luciferase. With the *Renilla*-Glo™ Luciferase Assay Reagent, no decrease in luminescence is seen with as much as 10% serum, and less than a 20% decrease in luminescence is seen with 2% solvent (Figure 5). At the highest concentrations of sera and solvent tested, there is no effect on the stability of the signal.

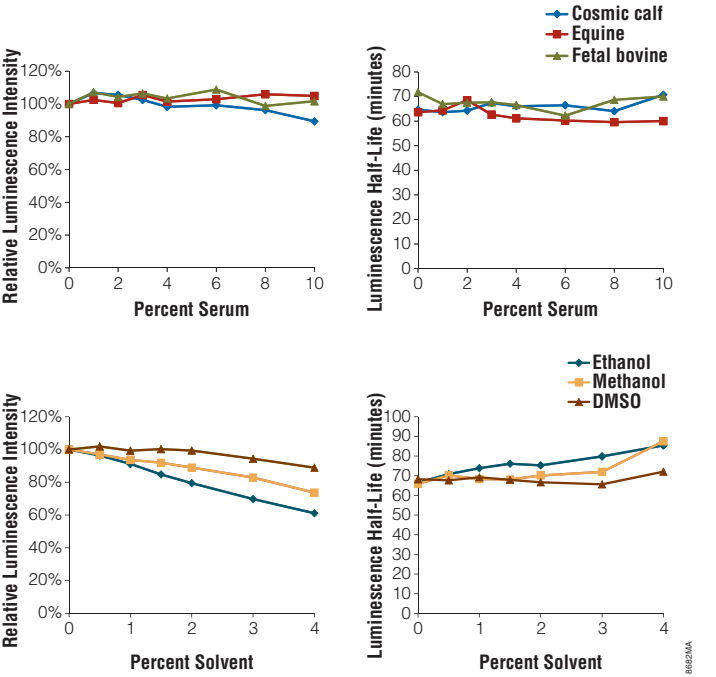

Figure 5. Effects of sera and solvent on luminescence intensity and signal stability. The *Renilla*-Glo™ Luciferase Assay Reagent was mixed (50µl + 50µl) with 70ng/ml of recombinant *Renilla* luciferase in DMEM without phenol red + 0.1% Prionex®. For sera, 0-10% of Cosmic calf serum, equine serum or fetal bovine serum was added to the reactions. For solvents, 0-4% of ethanol, methanol or DMSO was added to the reactions. The reactions were monitored as described in Figure 3. The relative intensity was determined from the signal measured 10 minutes after reagent addition and normalized to media without sera or solvent addition. The half-life was calculated from the decay of the signal from 10 to 75 minutes.

5.D. Compatibility with Robotic Platforms

To demonstrate that the *Renilla*-Glo™ Luciferase Assay can be used on a robotic platform in a 384-well format, an enzyme titration with two different plates at two different volumes was conducted (Figure 6). In each case, the R^2 value for the correlation was 0.9999.

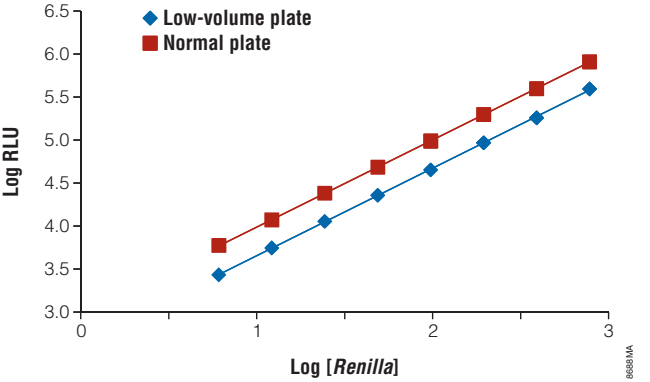

Figure 6. Detection of *Renilla* luciferase in a 384-well format on a robotic platform. A GST-*Renilla* construct was added to DMEM without phenol red at 780ng/ml and diluted in the same media. The enzyme was mixed with the *Renilla*-Glo™ Luciferase Assay Reagent using a Tecan Te-MO™ dispenser, the plates were shaken on a Union Scientific Corporation Horizontal Electromagnetic Microplate Medium Double Shaker Cat.#9759-RC for 1 minute and incubated at 21°C. Luminescence was read on a Tecan GENiosPro® at 12 to 14 minutes. A low volume 384-well plate (Corning Cat.# 3674) was used for the 3 + 3µl experiment, and a normal 384-well plate (Nunc Cat.# 165195) was used for the 10 + 10µl experiment.

[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.

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