Renilla Luciferase Assay System

INSTRUCTIONS FOR USE OF PRODUCTS E2810 AND E2820.

Quick PROTOCOL

Renilla Luciferase Assay Protocol

Before You Begin

1. Prepare 1X *Renilla* Luciferase Assay Lysis Buffer immediately before performing assays by diluting one volume of 5X *Renilla* Luciferase Assay Lysis Buffer into four volumes of distilled water. Mix well.
2. Follow the procedure in Section III.B (of TM055) for lysis of cells in a multiwell plate, or the procedure in Section III.C for lysis of cells grown in conventional culture dishes. Wait 15 minutes for cell lysis to occur.
3. Prepare *Renilla* Luciferase Assay Reagent: Add 1 volume of 100X *Renilla* Luciferase Assay Substrate to 100 volumes of *Renilla* Luciferase Assay Buffer in a glass or siliconized polypropylene tube. Prepare 100µl of Reagent per sample assayed.

For a manual luminometer:

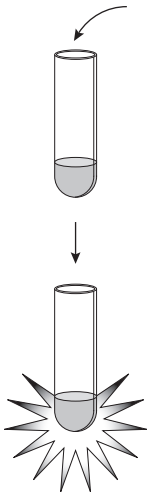
1. Add 100µl of *Renilla* Luciferase Assay Reagent to the luminometer tube.
2. Add 20µl of cell lysate. Mix quickly by flicking the tube or vortexing for 1–2 seconds.
3. Place the tube in a luminometer and initiate measurement. Luminescence should be integrated over 10 seconds with a 2-second delay.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

For a luminometer with a single reagent injector:

1. Format the luminometer to dispense 100µl. Prime the injector with *Renilla* Luciferase Assay Reagent.
2. For each reaction, add 20µl of cell lysate to an individual luminometer tube or to the wells of a multiwell plate.
3. Place the samples in the luminometer. Initiate dispensing of 100µl of Reagent per tube or plate well, then immediately initiate luminescence measurement of the sample. Luminescence is normally integrated over 10 seconds with a 2-second delay. Other read times may also be used.
4. If the luminometer is not connected to a printer or computer, record the luciferase activity measurements.

For additional protocol information see Technical Manual #TM055, available online at:
www.promega.com

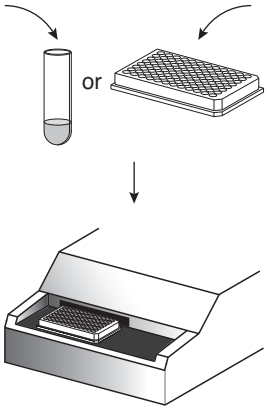
For manual luminometers:



Add 100µl of *Renilla* Luciferase Assay Reagent to tube. Then add 20µl of cell lysate and mix.

Measure luminescence.

For luminometers with single reagent injectors:



Add 20µl of cell lysate to a tube or plate well.

Place tube or plate in the luminometer. Initiate dispensing of 100µl of Reagent and luminescence measurement.

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phRL Synthetic *Renilla* Luciferase Reporter Vectors

The phRL *Renilla* control reporter vectors consists of seven vectors in which a synthetic *Renilla* luciferase gene sequence is used 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 phRL *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.

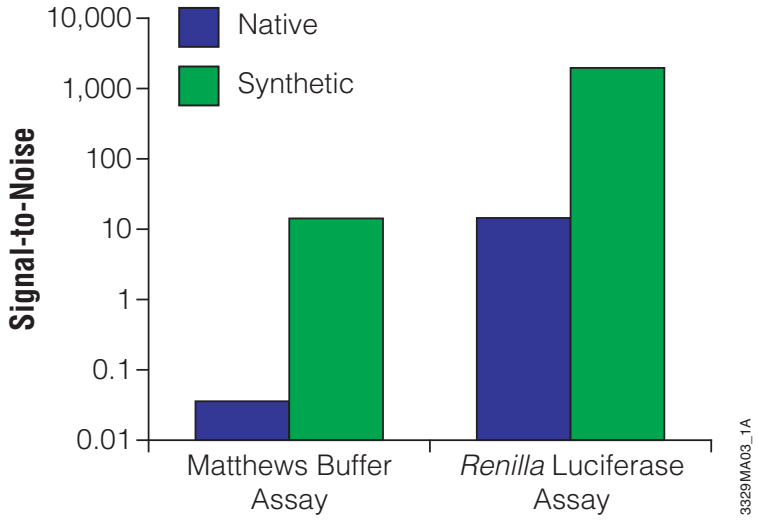

Figure 1. The *Renilla* Luciferase Assay System was used to measure expression from Promega's first generation of *Renilla* vectors containing the native *Renilla* gene and from the more recent phRL Vectors containing the synthetic *Renilla* gene. *Renilla* luciferase, expressed in CHO cells transfected 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 as follows. CHO cells containing *Renilla* luciferase were lysed in *Renilla* Luciferase Assay Lysis Buffer or Matthews Lysis Buffer (as described in Figure 2, TM055). Aliquots (20µl) of lysate containing the native or synthetic gene were added to 100µl of *Renilla* Luciferase Assay Reagent or Matthews Buffer. 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) from Promega.

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