



***Renilla* Luciferase Assay Kit**

Catalog Number: 30004-1 (150 assays)
30004-2 (1000 assays)

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Description

Renilla Luciferase has been used as a reporter gene for studying gene regulation and function in vitro and in vivo^{1,2}. Recently, *Renilla* luciferase has been widely used in multiplex transcriptional reporter assays or as a normalizing transfection control for *Firefly* luciferase assay^{2,3}. *Renilla* luciferase, a monomeric 36,000 Dalton protein, catalyzes coelenterazine oxidation by oxygen to produce light⁴ (Figure 1). The enzyme does not require post-translational modification for its activity and may function as a genetic reporter immediately following translation. Coelenterazine native is the natural substrate for *Renilla* luciferase. However, over a dozen of coelenterazine analogs have been synthesized, many of which are now commercially available from Biotium. These coelenterazine analogs all function as substrates for *Renilla* luciferase with different properties in term of emission wavelength, cell membrane permeability, and quantum efficiency. Coelenterazine also emits light from enzyme-independent oxidation, a process known as autoluminescence. The autoluminescence is enhanced by superoxide anion and peroxyxynitrite in cells and tissues. Biotium's *Renilla* Luciferase Assay Kit is designed to provide a simple and sensitive method of detecting *Renilla* luciferase. Through utilizing a special coelenterazine derivative and buffer formulation, this assay kit is designed to yield reliable, linear results with minimal autoluminescence background and superior sensitivity (Figure 2).

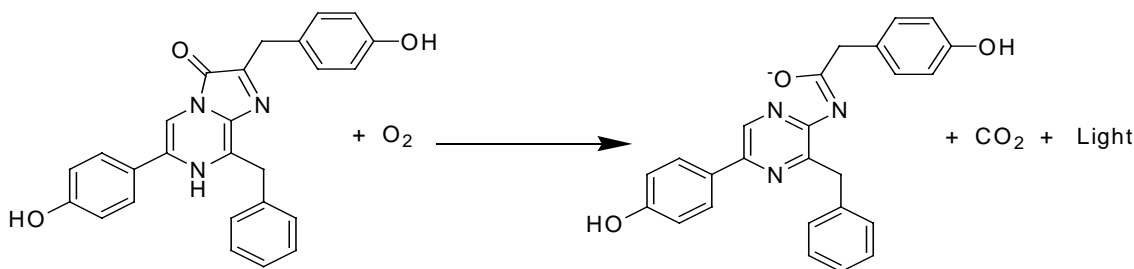


Figure 1. Bioluminescent reaction catalyzed by *Renilla* luciferase.

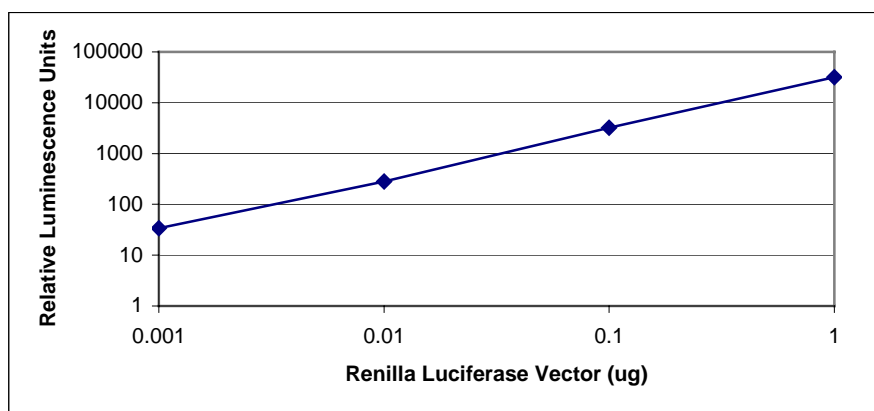


Figure 2. Dose response curve of transfected *Renilla* Luciferase genes. PC3 cells were transfected with 0.001ug, 0.01ug, 0.1ug, and 1ug pRL-CMV vector (Promega) encoding *Renilla* luciferase using Fugene 6 (Roche) in 6-well cell culture dishes. pGL2 Basic vector (Promega) was used as a control and for normalizing total DNA vector level to 1ug per transfection. Twenty-four hours after transfection, cells were harvested using 500μL lysis buffer contained in Biotium's *Renilla* Luciferase Assay Kit. To assay luciferase activity, 20μL of lysate from each sample was then added to 100μL of Assay buffer plus Enhancer contained in Biotium's *Renilla* Luciferase Assay Kit. Luminescence was measured on a luminometer (Turner Designs). Light emission was integrated over 10 seconds without delay.

Product Components

Renilla Luciferase Assay Kit, 30004-1 (150 assays)

Catalog Number	Component
99924	3 x Coelenterazine (100x) lyophilized
99911	10 mL 5X Passive Lysis Buffer
99913	10 mL <i>Renilla</i> Luciferase Assay Buffer
99915	10 mL <i>Renilla</i> Luciferase Assay Enhancer

Renilla Luciferase Assay Kit, 30004-2 (1000 assays)

Catalog Number	Component
99910	1 x Coelenterazine (100x) lyophilized
99912	30 mL 5X Passive Lysis Buffer
99914	50 mL <i>Renilla</i> Luciferase Assay Buffer
99916	50 mL <i>Renilla</i> Luciferase Assay Enhancer

Storage Conditions

Store the *Renilla* Luciferase Assay Kit at -20°C . The components of the kit are stable at -20°C for three months and at -70°C for up to six months. *Renilla* luciferase assay solution (Assay Buffer + Coelenterazine) should be prepared fresh for each use and used within 2 hours for best results. Avoid repeated freeze-thaw cycles. Aliquot *Renilla* Luciferase Assay buffer and Enhancer for storage if necessary.

Preparation of Cell Lysates

A. Preparation of 1X Passive Lysis Buffer

1X Passive Lysis Buffer is prepared by adding 1 volume of 5X Passive Lysis Buffer to 4 volumes of distilled water and mixing well. The 1X Passive Lysis Buffer may be stored at 4°C for up to one month. Store the 5X Passive Lysis Buffer at -20°C .

B. Lysis of Cells Cultured in Multiwell Plates

1. 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. Add 1X Passive Lysis Buffer using the volume recommended below for each type of well:

Wells/plate	Lysis buffer/well
6 well	500 μL
12 well	250 μL
24 well	100 μL
48 well	65 μL
96 well	20 μL

NOTE for Cat# 30004-2: Sufficient Passive Lysis Buffer is provided to perform 1000 assays with cells grown in 96 – 24 well plates. For applications requiring more lysis buffer (e.g. $>100\ \mu\text{L/well}$), additional 5X Passive Lysis Buffer (Cat. # 99912, 30 mL) may be purchased separately.

2. 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 Passive Lysis Buffer. Rock the culture plates at room temperature for 15 minutes.

Note: Cultures that are overgrown are often more resistant to complete lysis and typically require an increased volume of Passive Lysis Buffer and/or an extended treatment period to ensure complete lysis and/or scraping cells off the culture plates.

3. Transfer the lysate to a tube or vial and place in 4°C for further assay. Although it is not necessary, the lysate can be cleared by centrifugation for 30 seconds at top speed in a refrigerated microcentrifuge and transferred into a new tube. Store lysates at -70°C if not for immediate use.

Renilla Luciferase Assay

A. Preparation of *Renilla* Luciferase Assay Solution

Prepare an adequate volume to perform the desired number of *Renilla* Luciferase Assays (50 μL reagent per assay). Resuspend 1 vial of Coelenterazine with 50 μL MeOH (for component **99924**)

or 1 vial of Coelenterazine with 1 mL MeOH (for component **99910**) to derive 100X stock. Add 1 volume of 100X Coelenterazine stock to 50 volumes of *Renilla* Luciferase Assay Buffer in a glass or siliconized polypropylene tube to derive *Renilla* Luciferase Assay Solution (2X Coelenterazine solution). *Renilla* Luciferase Assay Solution (Coelenterazine + Buffer) should be prepared fresh and used within 2 hours. Store the remaining 100X Coelenterazine stock at -20°C.

B. Standard Protocol

For manual luminometer:

1. Set up luminometer with appropriate parameters (delay time, integration time and sensitivity, etc.).
2. Add 20µL of cell lysate into a luminometer tube.
3. Add 50µL of *Renilla* Luciferase Assay Enhancer into the tube, flick the tube a few times for thorough mixing.
3. Add 50µL of *Renilla* Luciferase Assay Solution (2X Coelenterazine solution) to the tube, flick the tube a few times for thorough mixing.
4. Place tube in luminometer and initiate measurement. Luminescence is normally integrated over 10 seconds without 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.
6. Discard the reaction tube, and proceed to the next *Renilla* Luciferase Assay.

For luminometer with injector:

1. Format the luminometer so that the injector dispenses 50µL. Prime the injector with *Renilla* Luciferase Assay Solution (2X Coelenterazine solution).
2. For each reaction, carefully add 20µL of cell lysate to an individual luminometer tube or to the wells of a multiwell plate.
2. Add 50uL *Renilla* Luciferase Assay Enhancer into each reaction.
3. Place the samples in a luminometer.
4. Initiate measurement. This action will cause *Renilla* Luciferase Assay Solution to be injected into the reaction vessel and the measurement to be subsequently taken. Luminescence is normally integrated over 10 seconds without pre-read delay. Other integration times may also be used.
5. Record the *Renilla* luciferase activity measurement.
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 Solution into the next well indicated on the luminometer plate.

Determination of Assay Backgrounds

The expression of a luciferase reporter is quantified by the luminescence produced above background levels. In most cases, because the background created by the reagent in the absence of *Renilla* luciferase is very small compared to the luciferase signal, 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 can be obtained by using lysate from untransfected cells or cells transfected with a control vector. The background luminescence can be subtracted from subsequent measurements of *Renilla* luciferase.

References

1. Bhaumik S. et al. (2004) Optical imaging of *Renilla* luciferase, synthetic *Renilla* luciferase, and firefly luciferase reporter gene expression in living mice. *J Biomed Opt.* **9**, 578-86.
2. Matijasevic Z. et al. (2001) Repair of sulfur mustard-induced DNA damage in mammalian cells measured by a host cell reactivation assay. *Carcinogenesis.* **22**, 661-4.
3. Nieuwenhuijsen BW. et al. (2004) A dual luciferase multiplexed high-throughput screening platform for protein-protein interactions. *J Biomol Screen.* **8**, 676-84.
4. Matthews, J.C., Hori, K. and Cormier, M.J. (1977) Purification and properties of *Renilla* reniformis luciferase. *Biochemistry* **16**, 85–91.