Biotium
Glowing Products for Science™

3159 Corporate Place
Hayward, CA 94545
www.biotium.com

Revised: January 3, 2014

Product Information

Renilla Luciferase Assay Kit

Catalog Number: 30004-T, 30004-1, 30004-2

Kit Contents

Component	30004-T 50 assays	30004-1 150 assays	30004-2 1000 assays
5X Passive Lysis Buffer	5 mL #99934	10 mL #99911	30 mL #99912
Renilla Luciferase Assay Buffer	5 mL #99935	10 mL #99913	50 mL #99914
Renilla Luciferase Enhancer	5 mL #99936	10 mL #99915	50 mL #99916
Coelenterazine (lyophilized)	1 vial (50 ug) #10110	3 vials (50 ug) #10110	4 vials (250 ug) #10110-2

Note: Sufficient passive lysis buffer is provided to perform the stated number of assays with cells grown in 96 – 24 well plates. For applications requiring more lysis buffer (e.g. >100 uL/well), additional 5X passive lysis buffer (Cat. # 99912) may be purchased separately.

Storage and Handling

Store the kit at –20°C or below. The components of the kit are stable at –20°C for three months and at –70°C for at least 6 months from date of receipt. Avoid repeated freeze-thaw cycles. Aliquot Renilla luciferase assay buffer and enhancer for storage if necessary. Renilla luciferase working solution (assay buffer + coelenterazine) should be prepared fresh for each use and used within 2 hours for best results.

Product 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 assays.[2,3] *Renilla* luciferase, a monomeric 36,000 Dalton protein, catalyzes coelenterazine oxidation by oxygen to produce light[4] (Figure 1). The enzyme does not require post-translational modification for its activity and may function as a genetic reporter immediately following translation. Coelenterazine also emits light from enzyme-independent oxidation, a process known as autoluminescence. The autoluminescence is enhanced by superoxide anion and peroxynitrite in cells and tissues. This assay kit utilizes a special buffer formulation designed to yield reliable, linear measurements of *Renilla* luciferase activity with minimal autoluminescence background and superior sensitivity (Figure 2). This kit is a flash-type luminescence assay, with signal half-life of about 2 minutes.

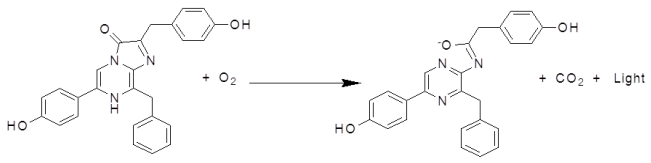

Figure 1. Bioluminescent reaction catalyzed by *Renilla* luciferase.

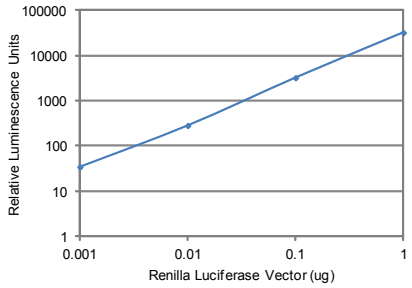

Figure 2. Dose response curve of transfected Renilla luciferase genes. PC3 cells were transfected with 0.001 ug, 0.01 ug, 0.1 ug, and 1 ug pRL-CMV vector (Promega) encoding Renilla luciferase using Fugene 6 (Roche) in 6-well cell culture dishes. pGL2 Basic vector (Promega) was used as a control and for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

Assay Protocols

Preparation of cell lysates

Preparation of 1X Passive Lysis Buffer

1. Prepare 1X Passive Lysis Buffer by adding 1 volume of 5X passive lysis buffer to 4 volumes of dH_2O and mixing well. 1X passive lysis buffer may be stored at 4°C for up to one month. Store 5X passive lysis buffer at –20°C.

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 uL
12 well	250 uL
24 well	100 uL
48 well	65 uL
96 well	20 uL

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. Biotium offers mini cell scrapers (cat. no. 22003) for harvesting lysates from 96-, 24-, and 48-well plates.

3. Transfer the lysate to a tube or vial. Optional: the lysate can be cleared by centrifugation for 30 seconds at top speed in a refrigerated microcentrifuge and transferred into a new tube. Place at 4°C for until ready to assay. Store lysates at -20°C or -70°C if assay will not be performed on the same day.

Continued next page

Renilla Luciferase Assay

Preparation of Renilla Luciferase Assay Solution

1. Prepare an adequate volume of working solution to perform the desired number of Renilla luciferase assays (50 uL working solution per assay). Thaw a bottle of Renilla luciferase assay buffer and pipette the desired volume into clean container.
2. Prepare 1 mg/mL coelenterazine:

   For kits 30004-T and 30004-1, dissolve one vial (50 ug) of coelenterazine (component 10110) in 50 uL MeOH.

   For kit 30004-2, dissolve one vial (250 ug) of coelenterazine (component 10110-2) in 250 uL MeOH.

   Note: For kits 30004-1 and 30004-2, the MeOH in the reconstituted coelenterazine can evaporate over time, so dissolve a new vial only when needed and store the vial sealed with Parafilm® M Sealing Film.

3. Add 1 volume of 1 mg/mL coelenterazine to 50 volumes of Renilla luciferase assay buffer to derive Renilla luciferase working solution. Renilla luciferase working solution (coelenterazine + Renilla luciferase assay buffer) should be prepared fresh and used within two hours. Store unused 1 mg/mL coelenterazine stock at -20°C.

Standard Protocol

For manual luminometer:

1. Set up luminometer with appropriate parameters (delay time, integration time and sensitivity, etc.).
2. Add 20 uL of cell lysate into a luminometer tube.
3. Add 50 uL of Renilla luciferase assay enhancer to the tube containing cell lysate and flick the tube a few times for thorough mixing.
4. Add 50 uL of Renilla luciferase working solution (assay buffer + coelenterazine) to the tube and mix quickly by vortexing or flicking the tube with a finger.
5. Place tube in luminometer and initiate measurement. Luminescence is normally integrated over 10 seconds without delay. Other integration times also may be used.
6. If the luminometer is not connected to a printer or computer, record the Renilla luciferase activity measurement.
7. Discard the reaction tube, and proceed to the next Renilla luciferase reaction.

For luminometer with injector:

1. Format the luminometer so that the injector dispenses 50 uL. Prime the injector with Renilla luciferase working solution (assay buffer + coelenterazine).
2. For each reaction, carefully add 20 uL of cell lysate to an individual luminometer tube or to the wells of a multiwell plate.
3. Add 50 uL Renilla luciferase assay enhancer into each reaction.
4. Place the samples in a luminometer.
5. Initiate measurement. This will cause Renilla luciferase working 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 also may be used.
6. Record the Renilla luciferase activity measurement.
7. If using a single tube luminometer, discard the reaction tube, and proceed to the next Renilla luciferase reaction. If using a plate luminometer, the luminometer will automatically begin injecting Renilla luciferase working solution into the next well indicated on the luminometer plate.

Determination of Background Luminescence

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.

Related Products

Catalog number	Product
30003	Firefly Luciferase Assay Kit
30005	Firefly & Renilla Dual Luciferase Assay Kit
30028	Steady-Luc Firefly HTS Assay Kit
30020	ATP-Glo Bioluminometric Cell Viability Assay
99912	5X Passive Lysis Buffer, 30 mL
22003	Mini Cell Scrapers, pack of 200

Please visit our website at www.biotium.com for information on our life science research products, including environmentally friendly EvaGreen® qPCR master mixes, fluorescent CF™dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.