



Firefly & Renilla Luciferase Assay Kit.

Catalog Number: 30005-1 (100 assays)
30005-2 (1000 assays)

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Description

Firefly luciferase is widely used as a reporter for studying gene regulation and function, and for pharmaceutical screening^{1,2}. It is a very sensitive genetic reporter due to the lack of any endogenous activity in mammalian cells or tissues^{3,4}. The *Firefly* luciferase is a 62,000 Dalton protein, which is active as a monomer and does not require subsequent processing for its activity. The enzyme catalyzes ATP-dependent D-luciferin oxidation by oxygen into oxyluciferin with emission of light centered on 560nm (Figure 1). As with many enzymes, *Firefly* luciferase follows Michaelis-Menten kinetics and, as a result, maximum light output is not achieved until the substrate and co-factors are present in large excess. When assayed under these conditions, light emitted from the reaction is directly proportional to the number of luciferase enzyme molecules. This *Firefly* luciferase assay kit is designed for detection and quantification of the *Firefly* luciferase reporter enzyme from cultured cells in a simple, efficient, and linear fashion (Figure 3).

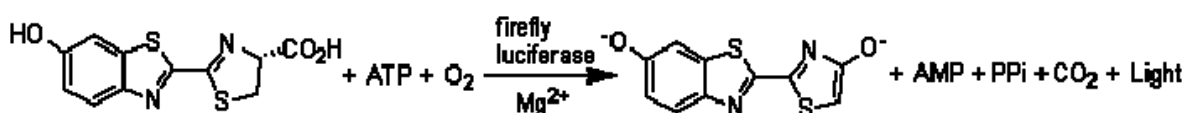


Figure 1. Bioluminescent reaction catalyzed by *Firefly* luciferase.

Renilla Luciferase has been used as a reporter for studying gene regulation and function in vitro and in vivo^{5,6}. Recently, *Renilla* Luciferase has been widely used in multiplex transcriptional reporter assays or as a normalizing transfection control for *Firefly* luciferase assay^{6,7}. *Renilla* luciferase, a monomeric 36,000 Dalton protein, catalyzes coelenterazine oxidation by oxygen to produce light⁸ (Figure 2). 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 peroxynitrite 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 3).

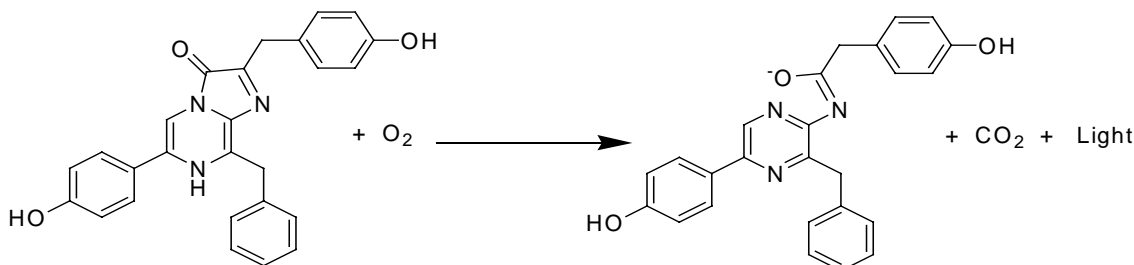


Figure 2. Bioluminescent reaction catalyzed by *Renilla* luciferase.

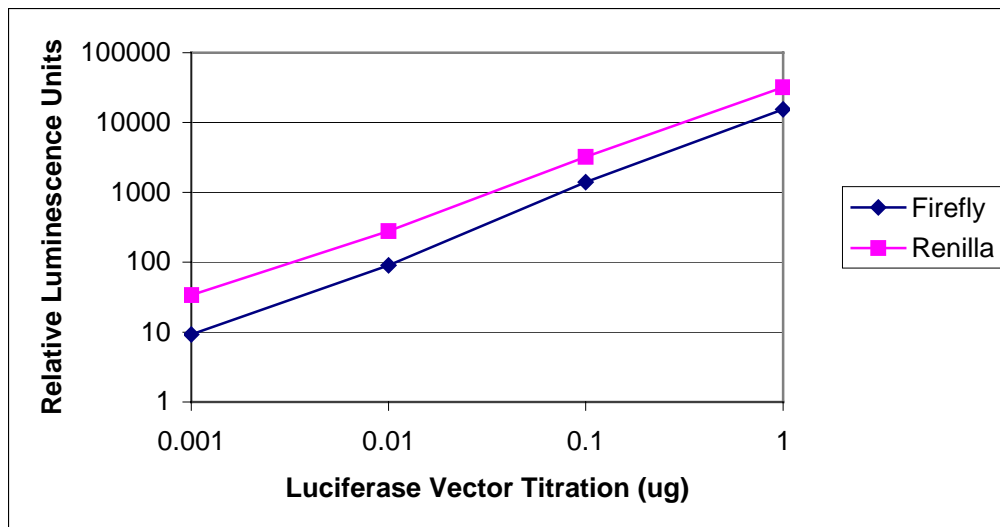


Figure 3. Dose response curve of transfected *Firefly* & *Renilla* Luciferase genes. PC3 cells were transfected with 0.001ug, 0.01ug, 0.1ug, and 1ug pFL-CMV and pRL-CMV vectors (Promega) encoding *Firefly* and *Renilla* luciferase genes by Fugene 6 in 6-well cell culture dishes. pGL2 Basic vector (Promega) was used as a control and for normalizing total DNA vector level to 2ug per transfection. Twenty-four hours after transfection, cells were harvested using 500µL lysis buffer contained in Biotium's *Firefly* & *Renilla* Luciferase Assay Kit. To assay luciferase activity, 20µL of lysate from each sample was then added to 100µL of *Firefly* Luciferase Assay Buffer or 100µL of *Renilla* Luciferase Assay Buffer plus Enhancer from *Firefly* & *Renilla* Luciferase Assay Kit. Luminescence was measured on a luminometer (Turner Designs). Light emission was integrated over 10 seconds without pre-read delay.

Product Components

Firefly & *Renilla* Luciferase Assay Kit, 30005-1 (100 assays)

Catalog Number	Component
99907	2 x 1mg D-Luciferin
99924	2 x Coelenterazine (100X) lyophilized
99932	1 x 15mL <i>Firefly</i> Luciferase Assay Buffer
99911	1 x 10mL Passive Lysis Buffer, 5X
99913	1 x 10mL <i>Renilla</i> Luciferase Assay Buffer
99915	1 x 10mL <i>Renilla</i> Luciferase Assay Enhancer

Firefly & *Renilla* Luciferase Assay Kit, 30005-2 (1000 assays)

Catalog Number	Component
99908	2 x 10mg D-Luciferin
99910	1 x Coelenterazine (100X) lyophilized
99909	1 x 100mL <i>Firefly</i> Luciferase Assay Buffer
99912	1 x 30mL Passive Lysis Buffer, 5X
99914	1 x 50mL <i>Renilla</i> Luciferase Assay Buffer
99916	1 x 50mL <i>Renilla</i> Luciferase Assay Enhancer

Storage Conditions

Store the *Firefly* & *Renilla* Luciferase Assay Kit at -70°C . *Firefly* luciferase assay solution (Assay Buffer + D-Luciferin) and *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.

Preparation of Cell Lysates

A. Preparation of 1X Passive Lysis Buffer

Passive Lysis Buffer 1X working solution is prepared by adding 1 volume of 5X Passive Lysis Buffer to 4 volumes of distilled water and mixing well. The 1X 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 growth medium from 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	500µL
12 well	250µL
24 well	100µL
48 well	65µL
96 well	20µL

Note for Cat.# 30005-2: 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 µL/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.

Firefly & Renilla Luciferase Assay

A. Preparation of Firefly Luciferase Assay Solution

1. Thaw a bottle of Firefly Luciferase Assay Buffer and pipette a desired volume (5mL or 50mL) from the bottle into a new container.

2. Dissolve the supplied D-luciferin with the above assay buffer at 0.2mg/mL concentration in a clean container. 5 mL assay solution is prepared from each 1 mg D-luciferin vial. Similarly, 50 mL assay solution is prepared from each 10 mg D-luciferin vial. Firefly Luciferase Assay Solution should be prepared fresh and used within a day.

Note: D-luciferin in Assay Buffer has limited stability. If you need less than 5mL or 50mL Luciferase Assay Solution as described in step 2, you may dissolve D-Luciferin in DI water as 10X or 50X stock solution and store it at -20°C or below for repeated use. The D-luciferin stock solution should be stable for at least one month, depending on the frequency of freeze-thaw cycle. A desired volume of the final Assay Solution can be prepared by diluting the stock solution with the supplied Assay Buffer to 0.2mg/mL D-luciferin.

B. Preparation of Renilla Luciferase Assay Solution

Prepare an adequate volume to perform the desired number of Renilla Luciferase Assays (50µL assay solution 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 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 two hours. Store the remaining 100X Coelenterazine stock at -20°C.

C. Standard Protocol

Note: This kit is designed for detecting the activity of *Firefly* and *Renilla* luciferases in a parallel fashion. Use manual mode or single injector mode for performing the assay as shown below. This kit does not fit dual luciferase reporter assay (DLR) mode using double injectors.

For manual luminometer:

1. Set up luminometer with appropriate parameters (delay time, integration time, sensitivity, etc.).
2. Add 20 μ L of cell lysate into a luminometer tube.
3. Add 100 μ L of *Firefly* Luciferase Assay Solution, mix quickly by flicking the tube with a finger 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 luciferase activity measurement.
6. Add 20 μ L of cell lysate into a new luminometer tube.
7. Add 50 μ L of *Renilla* Luciferase Assay Enhancer into the tube, flick the tube a few times for thorough mixing.
8. Add 50 μ L of *Renilla* Luciferase Assay Solution (2X Coelenterazine solution) to the tube, flick the tube a few times for thorough mixing.
9. Place tube in luminometer and initiate measurement. Luminescence is normally integrated over 10 seconds without delay. Other integration times may also be used.
10. If the luminometer is not connected to a printer or computer, record the luciferase activity measurement.
11. Discard the reaction tube, and proceed to the next Luciferase Assay.

For luminometer with injector:

Firefly Luciferase Assay

1. Format the luminometer so that the injector dispenses 100 μ L. Prime the injector with *Firefly* Luciferase Assay Solution.
2. For each reaction, carefully add 20 μ L 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 *Firefly* 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 *Firefly* luciferase activity measurement.

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

Renilla Luciferase Assay

1. Format the luminometer so that the injector dispenses 50 μ L. Prime the injector with *Renilla* Luciferase Assay Solution.
2. For each reaction, carefully add 20 μ L of cell lysate to an individual luminometer tube or to the wells of a multiwell plate.
2. Add 50 μ L *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 Background for *Renilla* Luciferase

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 low, this luciferase activity is directly proportional to total luminescence. However, when measuring very small amounts of *Renilla* 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

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