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Product Information

Firefly & *Renilla* Luciferase Single Tube Assay Kit

Catalog Number: PR300008

Kit Contents

Component	150 assays
1X Passive Luciferase Lysis Buffer 2.0	15 mL* 99821-15mL
Firefly Luciferase Assay Buffer 2.0	15 mL 99815-15mL
D-Luciferin	3 x 1 mg 99907
Renilla Luciferase Assay Buffer 2.0	15 mL 99816-15mL
Aquaphile™ Coelenterazine	3 x 200 ug 10126-200ug

* Enough lysis buffer is provided to perform the stated number of assays with cells grown in culture plate sizes ranging from 96-well to 24-well plates.

Storage and Handling

Store the kit at -80°C. Firefly and *Renilla* Assay Buffers are stable at -80°C for at least six months from date of receipt. Other components are stable at -20°C or below for at least six months from date of receipt. Kit components and stock solutions of D-Luciferin and Aquaphile Coelenterazine in water are stable to at least 5 freeze/thaw cycles.

Product 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 absence of endogenous luciferase activity in mammalian cells or tissues.[3,4] Firefly luciferase, a monomeric 62,000 Dalton protein, catalyzes ATP-dependent D-luciferin oxidation to oxyluciferin to produce light (Figure 1A).

Renilla luciferase has been used as a reporter gene for studying gene regulation and function in vitro and in vivo.[5,6] It commonly is used in multiplex transcriptional reporter assays or as a normalizing transfection control for firefly luciferase assays.[6,7] *Renilla* luciferase, a monomeric 36,000 Dalton protein, catalyzes coelenterazine oxidation by oxygen to produce light[8] (Figure 1B).

The Firefly & *Renilla* Luciferase Single Tube Assay allows measurement of Firefly and *Renilla* luciferase activity in the same sample with high sensitivity and linearity. Firefly luciferase activity is measured first, then *Renilla* Luciferase Assay Buffer 2.0 is added to simultaneously quench firefly luciferase activity and measure *Renilla* luciferase activity. *Renilla* Luciferase Assay Buffer 2.0 quenches the firefly luciferase activity to the level of untransfected cells, allowing sequential measurement of firefly and *Renilla* luciferase activity in the same sample (Figure 2). The assay has a wide linear range and high sensitivity for both firefly and *Renilla* luciferases (Figure 3). This is a flash-type assay that requires luminescence to be measured immediately after adding the detection reagents to the luciferase sample. Firefly signal decays over the course of about 12 minutes, while Renilla signal decays over the course of about 2 minutes, although this may vary depending on enzyme levels.

The Firefly & *Renilla* Luciferase Single Tube Assay features Biotium's water-soluble Aquaphile™ coelenterazine. It also includes our new 1X Passive Lysis Buffer 2.0, which is ready-to-use without dilution for cell lysis. The buffer also can be used to dilute recombinant firefly or *Renilla* luciferase enzymes without the need to add BSA to stabilize the enzymes.

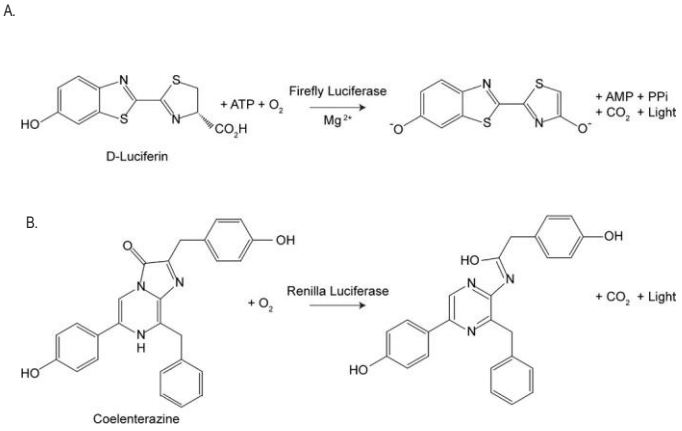

Figure 1. Bioluminescent reactions catalyzed by firefly luciferase (A) and *Renilla* luciferase (B).

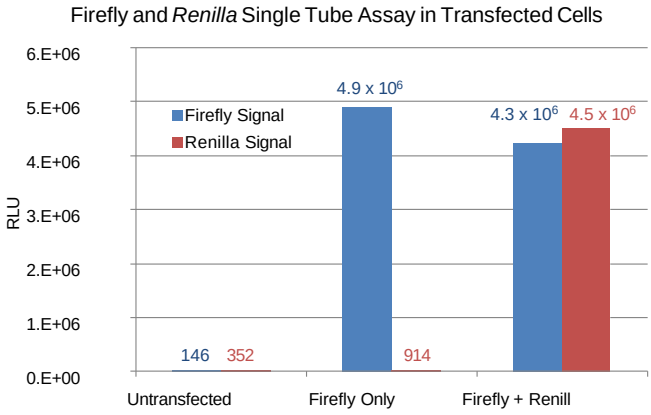

Figure 2. Example of Firefly & *Renilla* Luciferase Single Tube Assay using lysates from untransfected HeLa cells or cells transfected with either firefly luciferase alone (Firefly Only) or co-transfected with firefly and *Renilla* luciferases (Firefly + Renilla). In cells transfected with firefly only, the *Renilla* signal is the residual firefly luminescence after adding *Renilla* working solution to the reaction. Luminescence was measured on a Promega Glomax® 20/20 single tube luminometer using the Dual Glo® program with integration time of 1 second.

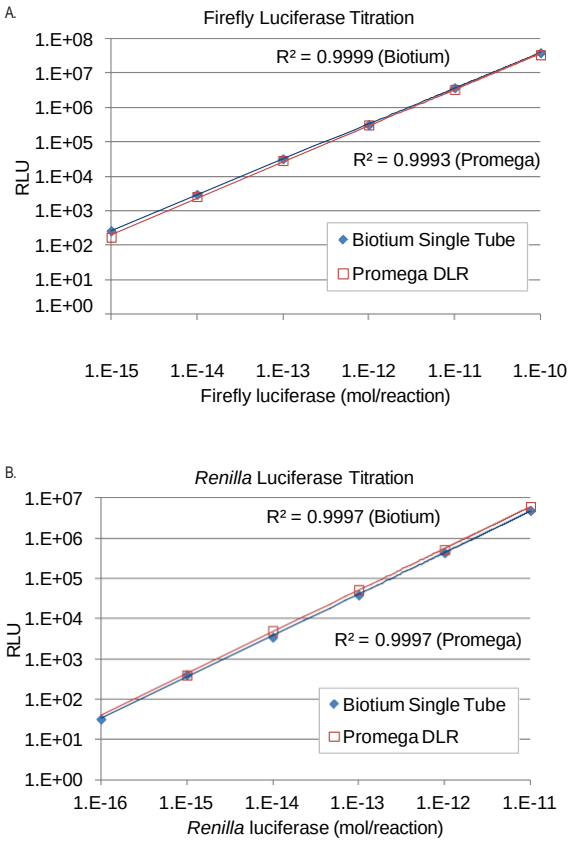

Figure 3. Comparison of linear ranges of Biotium's Firefly & *Renilla* Single Tube Assay and Promega's Dual Luciferase® Reporter Assay System (DLR™). Luciferase reactions were performed according to manufacturers instructions using titration curves of (A) purified recombinant QuantiLum® recombinant firefly luciferase (Promega) or (B) purified recombinant *Renilla* luciferase (Ray Biotech). Luciferase enzymes were diluted in 1X passive lysis buffer from the assay kit tested. Background from reagents without enzyme added was subtracted from luminescence values. Both assay systems showed comparable sensitivity and dynamic range for firefly and *Renilla* luciferases.

Assay design considerations

Co-transfection experiments

The co-transfection of a control vector together with a reporter vector can suppress expression of the reporter gene[9]. Therefore, preliminary co-transfection experiments should be carried out to determine the optimal ratio of experimental and control plasmids to reliably measure luminescence values above background while minimizing interference in gene expression between vectors. Total mass of transfected DNA also can affect reporter transfection efficiency and/or gene expression, therefore the total mass of DNA used to transfect each sample should be the same. Control DNA (i.e., empty vector) can be used to balance the total amount of DNA per sample.

Recombinant luciferase enzymes

Purified recombinant firefly and *Renilla* luciferase enzymes are available commercially, and can be useful positive controls for luciferase assays. Unlike our earlier formulation of passive lysis buffer (5X Passive Lysis Buffer, catalog no. 99923), our new 1X Passive Lysis Buffer 2.0 can be used with recombinant firefly or Renilla luciferase without the need to add BSA or other enzyme stabilizer. Note that 1X Passive Lysis Buffer 2.0 contains protein stabilizers that may affect results if the buffer is used in a protein quantitation assay.

Determination of assay background

The expression of a luciferase reporter is quantified by the luminescence produced above background levels. In most cases, background created by the reagent in the absence of luciferase is very low compared to signal with luciferase. However, when measuring low levels of luciferase activity, it is important to subtract the background signal from untransfected cells or cells transfected with a negative control vector from measurements of luciferase activity.

The contribution of residual firefly luciferase activity to *Renilla* background can be determined by performing the dual luciferase assay in cells transfected with firefly luciferase alone (without *Renilla* luciferase) to determine the apparent *Renilla* signal contributed by residual firefly activity. The ratio of firefly and *Renilla* expression levels should be optimized to minimize contribution of residual firefly luminescence to *Renilla* background.

Quick Start Protocol
This is a general assay overview for quick reference. See the Assay Protocol on page 3 for complete instructions.

1. Lyse cells in 1X Passive Lysis Buffer 2.0. Note: buffer is ready to use without dilution.
2. Aliquot 20 uL of cell lysate to each reaction tube.
3. Prepare firefly and *Renilla* working solutions.
4. Add 100 uL firefly working solution to reaction tube and mix by pipetting up and down.
5. Immediately read firefly luminescence.
6. Add 100 uL *Renilla* working solution to the same reaction tube and mix.
7. Immediately read *Renilla* luminescence.
8. Discard reaction tube and proceed to the next sample.

Assay Protocol

Preparation of cell lysates

Note: 1X Passive Lysis Buffer 2.0 is ready to use without dilution.

1. Remove the growth medium from the cultured cells and gently wash the cells once with a sufficient volume of phosphate buffered saline (PBS) to cover the surface of the culture vessel. Remove the PBS and add 1X Passive Lysis Buffer 2.0 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.

   Note: 1X Passive Lysis Buffer 2.0 contains protein stabilizers that may affect results of protein quantitation assays.

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 until ready to assay. Lysates can be stored at -20°C or -80°C for up to two weeks.

Preparation of Firefly Working Solution

1. Thaw Firefly Luciferase Assay Buffer 2.0 at room temperature.
2. Prepare 10 mg/mL D-luciferin stock solution. For component 99907 (1 mg), add 100 uL water to the vial and mix. For component 99908 (10 mg), add 1 mL water to the vial and mix. The stock solution can be stored for at least 6 months at -20°C or below, and is stable to up to 5 freeze/thaw cycles.
3. Prepare enough firefly working solution to perform the desired number of assays (100 uL working solution per assay). Dilute D-luciferin (10 mg/mL) in assay buffer at a ratio of 1:50. For example, add 20 uL D-luciferin stock solution to 1 mL firefly assay buffer.

   Note: For best results, working solutions (assay buffer with substrate) should be prepared fresh before each use, and used within 3 hours of preparation. Firefly working solution activity decreases ~10% after 3 hours and ~25% after 5 hours at room temperature.

Preparation of *Renilla* Working Solution

1. Thaw *Renilla* Luciferase Assay Buffer 2.0 at room temperature.
2. Prepare 2 mg/mL Aquaphile™ coelenterazine stock solution. For component 10126-200ug, add 100 uL water to the vial and mix. For component 10126-4mg, add 2 mL water to the vial and mix. The stock solution can be stored for up to 3 months at -20°C or below, and is stable to up to 5 freeze/thaw cycles.
3. Prepare enough *Renilla* working solution to perform the desired number of assays (100 uL working solution per assay). Dilute Aquaphile™ coelenterazine (2 mg/mL) in *Renilla* Luciferase Assay Buffer 2.0 at a ratio of 1:50. For example, add 20 uL Aquaphile™ coelenterazine stock solution to 1 mL assay buffer.

   Note: For best results, working solutions (assay buffer with substrate) should be prepared fresh before each use, and used within 3 hours of preparation. *Renilla* working solution activity is stable for up to 3 hours, but background increases up to 60% after 5 hours at room temperature.

Firefly & *Renilla* Luciferase Single Tube Assay

The protocol below is for manual assay using a single-tube luminometer. If your luminometer is equipped with automatic injectors, they may be used to dispense one or both working solutions into each luminometer tube or well of a multiwell plate according to the instructions for your instrument.

1. Set up luminometer with parameters recommended for your instrument for dual luciferase assay. We routinely use integration time of 1 second.
2. Add 20 uL of cell lysate into a reaction tube that is compatible with your luminometer.
3. Add 100 uL of firefly working solution to the reaction tube and mix by pipetting up and down several times.

   Note: Do not vortex the tube, which could cause the firefly reaction mix to coat the upper part of the tube and not effectively mix with the *Renilla* working solution in step 5.
4. Immediately place tube in luminometer and record the firefly luminescence measurement.
5. Add 100 uL of *Renilla* working solution to the same reaction tube and mix by pipetting or vortexing.
6. Immediately place tube in luminometer and record the *Renilla* luminescence measurement.
7. Discard the reaction tube, and proceed to the next reaction.

   Note: *Renilla* working solution can be used to measure *Renilla* luciferase activity in the absence of firefly luciferase, but for direct comparison to samples with both Firefly and *Renilla* luciferases, you should first add firefly working solution before adding *Renilla* working solution so the final assay volume remains constant between samples. For determination of *Renilla* activity only, firefly working solution can be omitted.

References

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