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Product Information

Firefly & Renilla Dual Luciferase Assay Kit

Catalog Number: 30005-T, 30005-1, 30005-2

Kit Contents

Component	30005-T 50 assays	30005-1 100 assays	30005-2 1000 assays
5X Passive Lysis Buffer	5 mL #99934	10 mL #99911	30 mL #99912
Firefly Luciferase Assay Buffer	5 mL #99933	15 mL #99932	100 mL #99909
D-Luciferin	1 x 1 mg #99907	2 x 1 mg #99907	2 x 10 mg #99908
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	2 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 kit is stable at –20°C for three months and at –70°C at least six months from date of receipt. Avoid repeated freeze-thaw cycles. Firefly and Renilla working solutions should be prepared fresh before each use. For best results, use Renilla luciferase working solution within 2 hours of preparation and use firefly luciferase working solution within one day of preparation.

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). This firefly luciferase assay is designed for simple and efficient quantitation of firefly luciferase reporter enzyme activity from cultured cells with high sensitivity and linearity (Figure 2). This is a flash-type firefly assay with a signal half-life of about 12 minutes.

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). Coelenterazine also emits light from enzyme-independent oxidation, a process known as autoluminescence. This assay kit utilizes a special buffer formulation designed to yield reliable, linear measurements of *Renilla* luciferase activity with minimal background and superior sensitivity (Figure 2). This is a flash-type *Renilla* assay with a signal half-life of about 2 minutes.

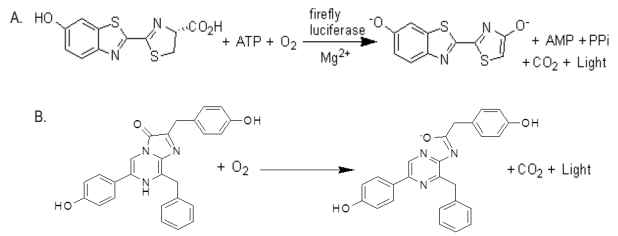

Figure 1. Bioluminescent reactions catalyzed by firefly luciferase (A) and *Renilla* luciferase (B).

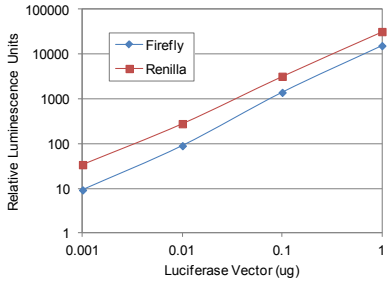

Figure 2. Dose response curve of transfected Firefly & Renilla Luciferase genes. PC3 cells in 6-well plates were transfected with 0.001-1 ug each pFL-CMV and pRL-CMV vectors (Promega) encoding firefly and Renilla luciferase genes using Fugene 6 (Roche). pGL2 Basic vector (Promega) was used as a control and for normalizing total DNA vector level to 2 ug per transfection. Twenty-four hours after transfection, cells were harvested using 500 uL lysis buffer. Luciferase activity in 20 uL of lysate from each sample was measured using the Firefly & 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

Note: This is a two-tube dual luciferase assay. Separate aliquots of lysate are required for the firefly and Renilla reactions. For the assay protocols listed below, 20 uL lysate is required for firefly luciferase measurement and 20 uL lysate is required for Renilla luciferase measurement, therefore 40 uL total lysate is required to measure both enzyme activities, equivalent to 2 wells of a 96 well plate.

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.

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Firefly & Renilla Luciferase Assay

Preparation of Firefly Luciferase Working Solution

1. Prepare an adequate volume of working solution to perform the desired number of firefly luciferase assays (100 uL working solution per assay). Thaw a bottle of firefly luciferase assay buffer and pipette a desired volume (5 mL or 50 mL) from the bottle into a clean container.
2. Dissolve the supplied D-luciferin in the firefly luciferase assay buffer from step 1 at a final concentration of 0.2 mg/mL. For kit 30005-1, dissolve one vial of D-luciferin (component 99907, 1 mg/vial) in 5 mL assay buffer. For kit 30005-2, dissolve one vial of D-luciferin (component 99908, 10 mg/vial) in 50 mL assay buffer. Firefly luciferase working solution (D-luciferin + firefly luciferase assay buffer) should be prepared fresh and used within a day.

   Note: D-luciferin in assay buffer has limited stability. If you need less than 5 mL or 50 mL luciferase working solution as described in step 2, you may dissolve D-luciferin in dH_2O 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 cycles. The required volume of working solution can be prepared by diluting the stock solution in firefly luciferase assay buffer to a final concentration of 0.2 mg/mL D-luciferin.

Preparation of Renilla Luciferase Working 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 a clean container.
2. Prepare 1 mg/mL coelenterazine:

   For kits 30005-T and 30005-1, dissolve one vial (50 ug) of coelenterazine (component 10110) in 50 uL MeOH. For kit 30005-2, dissolve one vial (250 ug) of coelenterazine (component 10110-2) in 250 uL MeOH.

   Note: For kits 30005-1 and 30005-2, 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

Note: This kit is designed for detecting the activity of firefly luciferase and Renilla luciferase in a parallel fashion. Use manual mode or single injector mode for performing the assay as shown below. This assay is not compatible with 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 uL of cell lysate into a luminometer tube.
3. Add 100 uL of firefly luciferase working solution and mix quickly by vortexing or flicking the tube with a finger.
4. Place tube in luminometer and initiate measurement. Luminescence is normally integrated over 10 seconds without delay. Other integration times also may be used.
5. If the luminometer is not connected to a printer or computer, record the luciferase activity measurement.
6. Add 20 uL of fresh cell lysate into a new luminometer tube.
7. Add 50 uL of Renilla luciferase assay enhancer into the tube and mix quickly by vortexing or flicking the tube with a finger.
8. 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.
9. Place tube in luminometer and initiate measurement. Luminescence is normally integrated over 10 seconds without delay. Other integration times also may 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 reaction.

For Luminometer with Injector

Firefly Luciferase Assay

1. Format the luminometer so that the injector dispenses 100 uL. Prime the injector with firefly luciferase working solution.
2. For each reaction, add 20 uL 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 will cause the firelfy working solution to be injected into the sample). Luminescence is normally integrated over 10 seconds without pre-read delay. Other integration times also may 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 reaction. If using a plate luminometer, the luminometer will automatically begin injecting firefly luciferase working solution into the next well indicated on the luminometer plate.

Renilla Luciferase Assay

1. Format the luminometer so that the injector dispenses 50 uL. Prime the injector with Renilla luciferase working solution.
2. For each reaction, 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 to each reaction.
4. Place sample in luminometer and initiate measurement (this will cause the Renilla working solution to be injected into the sample). Luminescence is normally integrated over 10 seconds without pre-read delay. Other integration times also may 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 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 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, and subtracted from subsequent measurements of Renilla luciferase.

References

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Related Products

Catalog number	Product
99912	5X Passive Lysis Buffer, 30 mL
22003	Mini Cell Scrapers, pack of 200
30003	Firefly Luciferase Assay Kit
30075	Firefly Luciferase Assay Kit (Lyophilized)
30004	Renilla Luciferase Assay Kit
30028	Steady-Luc Firefly HTS Assay Kit
30020	ATP-Glo Bioluminometric Cell Viability Assay

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