GenDEPOT

LucyQ Duo-Luciferase (Firefly & Renilla) Assay Kit

L0013

Storage

Store at -20°C for long term.

Contents

- Product Manual
- LucyQ Cell Lysis Buffer(10X) L0013CLB
- LucyQ FLuc Assay Buffer(5X) L0013FAB
- LucyQ Firefly Luc Substrate(100X) L0013FLS
- LucyQ RLuc Assay Buffer(5X) L0013RAB
- LucyQ Renilla Luc Substrate(100X) L0013RLS

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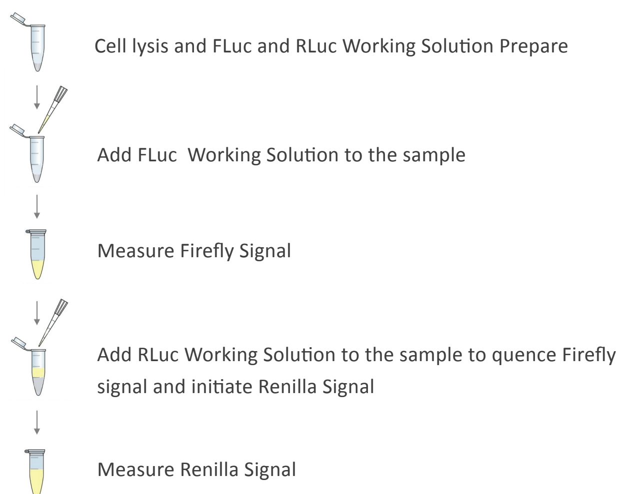
Shipping Condition

Ship with dry ice.

Introduction

Firefly and Renilla luciferases are widely used as reporter genes for studying gene regulation and function, and for pharmaceutical screening. Renilla Luciferase is often used in conjunction with Firefly Luciferase as a normalizing transfection control or for multiplex transcriptional reporter assays. As with many enzymes, Firefly luciferase and Renilla luciferase follow Michaelis-Menten kinetics and thus maximum light output is not achieved until substrates (above the Km) and co-factor 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. LucyQ Duo-Luciferase(Firefly & Renilla) Assay kit is designed for detection and quantification of Firefly and Renilla luciferase reporter enzymes from cultured cells in a simple, efficient and linear fashion.

Schemati Overview



LucyQ Duo-Luciferase Assay Principles

Firefly and Renilla luciferases have been widely used as co-reporters for normalization studies because both assay are quick, easy and sensitive. Firefly and Renilla luciferases are ideal co-reporters because they have distinct evolutionary origins and very different enzyme structures and substrates. This renders cross-reactivity obsolete. Firefly (Photinus pyralis) luciferase has been proven to be an ideal reporter for monitoring both promoter activity and post-transcriptional regulation in the control of gene expression.

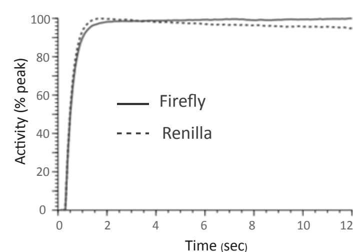


Figure 1. Luminescent signals generated in the LucyQ Duo-Luciferase Assay Kit.

Renilla (Renilla reniformis) luciferase is a 36 kDa monomeric protein, which requires no post-translational processing. Threfore, it can function as a real-time transcription reporter.

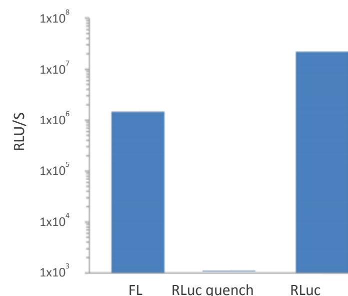


Figure 2. Firefly luciferase activity is quenched with the addition of RLuc. HEK 293 cells were transfected with Promega pGL4.13/pGL4.75 reporter vectors for 48 hours.

The firefly luciferase activity was measured as described in the procedure. Afterwards, 1× RLuc buffer (without or with substrate) was add to the wells, followed by count reading in a luminometer.

About 99.9% of firefly luciferase activity was quenched (middle column).

Product Features

High sensitivity.
The reagents have been developed so that the signals for Firefly and Renilla luciferases exhibit greatest sensitivity.

Versatility.
The system has been designed for assays with many different eukaryotic (vertebrates, lower invertebrates) cells using micro-plates or single-tube luminescence readers.

Low background.
The system produces very limited background luminescence. No subtraction is required from readings.

Simplicity.
Renilla luciferase buffer contains the quencher for Firefly luciferase activity. This allows for a quick Glow and Stop-N-Glow two-step assays.

Reproducibility.
This system is designed to yield reliable, linear results for a concentration range over several orders of magnitude.

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Preparation of Cell Lysates using LucyQ Cell Lysis Buffer

The LucyQ Cell Lysis Buffer is supplied as a 10X concentrate. It may show turbid after thawing which won't affect the luciferase assays. Vortex 3-5 sec after thawing, and prepare a sufficient quantity of the 1X working concentration by adding 1 volume of LucyQ Cell Lysis Buffer(10X) to 9 volumes of distilled water and mix. The diluted LucyQ Cell Lysis Buffer(1X) may be stored at -20°C for 1-2 months; however, we recommend preparing the volume of LucyQ Cell Lysis Buffer(1X) required just before use.

A. Lysis of cells cultured in multi-well plates.

1. Determine transfection parameters (i.e., plated cell density and subsequent incubation time) such that cells are 80-95% confluent at the desired time of lysate preparation. Remove the growth medium from the cultured cells, and gently apply a sufficient volume of phosphate buffered saline (PBS) to wash the surface of the culture vessel. Swirl the vessel briefly to remove detached cells and residual growth medium. Completely remove the rinse solution before applying LucyQ Cell Lysis Buffer.

2. Dispense into each culture well the minimum volume of LucyQ Cell Lysis Buffer(1x) required to completely cover the cell monolayer. The recommended volumes of LucyQ Cell Lysis II Buffer(1X) to add per well are as follows:

Culture Plate	Lysis Buffer(1X)
6 well plate	500 ul
12 well plate	250 ul
24 well plate	100 ul
48 well plate	65 ul
96 well plate	20 ul

Note: The LucyQ Cell Lysis Buffer(10X) provided in the kit is sufficient for directly lysing cells in 24-, 48- or 96-well culture plates. If a 6- well or 12-well plates are used, we recommend either purchasing more LucyQ Cel Lysis Buffer(10X) or harvesting cells by scraping or trypsinization according to the procedures in B below.

3. 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 LucQ Cell Lysis Buffer(1X). Rock the culture plates at room temperature for 10-15 minutes.

Note: If cell clumps appear, pipetting several times could be helpful to disperse the cells. Alternatively, collect the cell lysates including cell clumps in tubes and vortex 5-10 sec after cooling down on ice, then 1 to 2 freeze-thaw cycles to accomplish complete lysis of cells. Overgrown cells are more resistant to complete lysis, and typically require an increased volume of LucQ Cell Lysis Buffer(1X) to ensure complete lysis.

LucyQ Duo-Luciferase Assay Principles

Note: The Firefly and Renilla luciferases contained in the cell lysates are stable for at least 30minutes at room temperature (22°C) and up to 2 hours on ice. –70°C is recommended for long-term storage. Subjecting cell lysates to more than 5 freeze-thaw cycles may result in gradual loss of luciferase reporter enzyme activities.

4. Transfer the lysate to a tube or vial for further handling and storage. Alternatively, reporter assays may be performed directly in the 96-well culture plate if the plates are compatible with the type of luminometer being used.

5. Proceed to Luciferase assays.

B. Lysis of cells in tubes.

1. For cells cultured in suspension, or cells harvested by scraping or trypsinization. Collect 1-2×10^5 cells in 1.5 mL tubes, rinse cells with 1mL of PBS buffer, spin at 500g for 5 minutes, and completely remove the rinse solution.

2. Add 50-100 μL of LucyQ Cell Lysis Buffer(1X) to make 2×10^3 cells/μL, Vortex 5-10 sec to completely disperse the cells, then 1 to 2 freeze-thaw cycles to accomplish complete lysis of cells.

3. Proceed to Luciferase assays.

Note: The Firefly and Renilla luciferases contained in the cell lysates are stable for at least 30 minutes at room temperature (22°C) and up to 2 hours on ice. –70°C is recommended for long-term storage. Subjecting cell lysates to more than 5 freeze-thaw cycles may result in gradual loss of luciferase reporter enzyme activities. 2×10^3 cells/μL in LucyQ Cell Lysis Buffer(1X) is good for the assay in normal transfected cells. If the cells have lower transfection efficiency or the promotor is very weak, you may increase the cell numbers. This LucyQ Cell Lysis Buffer is optimized for compatibility with the following FLuc and RLuc detection assays. If other cell lysis buffers are used, the signal strength of the luciferase could be affected.

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Preparation of FLuc and RLuc Assay Working Solution

Note1. LucyQ FLuc and RLuc Assay Buffers are stable at –20°C for at least 6 months. Freezing and thawing the reagents 5-6 cycles won't affect the activity of the luciferases. Aliquotting is recommended if more freeze-thaw cycles are required.

Note2. Working Solutions (Buffers containing Substrates) are stable at room temperature for 1-2 hours. Prepare only the required amount of Working Solution before use.

Note3. Light intensity is a measure of the rate of catalysis by the luciferases, and is therefore; temperature sensitive. The temperature optimum for the activity of both luciferases is approximately room temperature (20–25°C), so it is important that the reagents be equilibrated to room temperature before beginning measurements. This kit is not designed for single luciferase detection. If using for detection of a single luciferase, the procedures for dual luciferase detection in this manual should still be strictly followed.

1. Thaw LucyQ FLuc Assay Buffer (5X) and LucyQ RLuc Assay Buffer (5X) thoroughly at room temperature, inverting the tube several times and then vortex for 3-5 seconds.

Note: Some pellets may appear in the LucQ RLuc Assay Buffer (5X) after thawing. It is important to completely dissolve the pellets before using. Incubation at 37°C for 5-10 minutes and more vortexing will be necessary to fully re-dissolve the pellets.

2. Dilute 1:5 in distilled water to make LucyQ FLuc Buffer(1X) and LucyQ RLuc Assay Buffer(1X). Prepare 100 µL of each Buffer for each reaction (well). Duplicates or triplicates for each sample are recommended. Example: If you have 5 samples in duplicated reactions, prepare 1 mL of LucQ FLuc Assay Buffer(1X) and LucQ RLuc Assay Buffer(1X). By diluting 0.2 mL of each 5X Buffers with 0.8 mL ddH_2O respectively. Preparing a little extra may be helpful to avoid buffer shortage caused by pipetting error.

3. Prepare the FLuc and RLuc Assay Working Solution (e.g.10 samples) by adding 10 µL of LucyQ Firefly Luc Substrate(100X) and Renilla Luc Substrate (100X) to 1 mL of LucyQ FLuc Assay Buffer(1X) and LucyQ Rluc Assay Buffer respectively. Mix well by inverting the tube several times.

4. Incubate at room temperature for 5 minutes (capped and protected from light) before adding to the samples.

Note: The RLuc Assay Working Solution will be used after reading the FLuc assay. It can be kept at room temperature as long as 1 hour if properly capped and protected from light.

Assay Procedure

1. Set up the luminometer. Follow the manual associated with your plate reader. Set the measurement for 1-2 seconds of integration.

2. Pipette the cell lysis samples (20 µL per well) into a 96-well white (opaque) or black plate, or luminometer tubes.

3. Add the FLuc Assay Working Solution to the samples. Gently pipette up and down mix the sample and assay solution. Do not vortex.

Note: If you have many samples and use 96-well plates, we recommend using a multi-channel pipette in order to reduce the time between additions of Assay Working Solution to each well. Auto-Injector: If using Injectors, follow the procedures described in the instrument's manual.

4. Proceed with the measurement.

Note: If using single luminometer tubes, make sure the processing times before the luminescence detection are identical for all samples.

5. Save the reading if the luminometer reader does not automatically record the readings.

6. Remove the plates or luminometer tubes.

7. Add the RLuc Working Solution to the plates or tubes from Step 6. Gently pipette up and down or tap the plate (tube) several times to mix the sample and assay solution. Do not vortex.

Note: If you have many samples and use 96-well plates, we recommend using a multi-channel pipette in order to reduce the time between additions of Assay Working Solution to each well. Auto-Injector: If using Injectors, follow the procedures described in the instrument's manual.

8. Proceed with the measurement.

Note: If using single luminometer tubes, make sure the processing times before the luminescence detection are identical for all samples.

9. Record the reading if the luminometer reader does not automatically save the readings.

10. Remove the plates or luminometer tubes.

11. Calculate the ratio of luminescence from the Firefly luciferase to the Renilla luciferase.

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Important Note

Because the luminescent signals are affected by assay conditions, raw results should be compared only between samples measured at the same time and using the same medium/serum combination. Incorporation of consistent control wells on each plate provides the ability to calculate a normalized Firefly luminescence/Renilla luminescence ratio for each sample well. This kit is not designed for single luciferase detection. If using for detection of a single luciferase, the procedure for dual luciferase detection in this manual still should be strictly followed. You may also purchase our single luciferase detection kits.

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