LucyQ Firefly Luciferase Assay Kit

L0010

Storage

Store at -20°C.

If stored properly, the product is stable at least 6 months.

Contents

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• LucyQ FL Cell Lysis Buffer(10X)	L0010CLB
• LucyQ FL Assay Buffer(5X)	L0010FAB
• LucyQ FL Substrate(100X)	L0010FLS

Introduction

LucyQ Firefly Luciferase Assay Kit is specifically designed for the simple and efficient quantification of firefly luciferase reporter enzyme activity from cultured cells with the highest degree of sensitivity, reliability, and linearity. This kit is a flash-type luminescent assay, and requires measurement immediately after adding the substrate to the sample. LucyQ Firefly Luciferase Assay Kit includes our improved 10X LucyQ FL Cell Lysis Buffer which provides a better working solution stability compared to other firefly assay kits.

Preparation of 1X LucyQ FL Cell Lysis Buffer

The LucyQ FL 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 FL Cell Lysis(10X) Buffer to 9 volumes of distilled water and mix. The diluted LucyQ FL Cell Lysis Buffer (1X) may be stored at -20°C for 1-2 months; however, we recommend preparing the volume of LucyQ FL Cell Lysis Buffer required just before use.

Preparation of Firefly Luciferase Working Solution (FLWS)

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

2. Dilute 1:5 in distilled water to make 1X LucyQ FL Assay Buffer. Prepare 100µL 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 1mL of 1X LucyQ FL Assay Buffer. By diluting 0.2mL of the 5X LucyQ FL Assay Buffers with 0.8mL ddH2O. Preparing a little extra may be helpful to avoid buffer shortage caused by pipetting error.

3. Prepare the Firefly Luciferase Working Solution(FLWS) (e.g.10 samples) by adding 10µL of LucyQ FL Substrate (100X) to 1mL of 1X LucyQ FL Assay Buffer. 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 : LucyQ FL Assay Buffer is 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. Aliquot is recommended if more freeze-thaw cycles are required.

Note : Firefly Luciferase Working Solution (FLWS) are stable at room temperature for 1-2 hours. Prepare only the required amount of Working Solution before use.

Note : 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 the 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 designed for single luciferase detection, and may not be used for dual luciferase detection.

Preparing Cell Lysates

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 Lysis Buffer.

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

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

Note : The LucyQ FL Cell Lysis Buffer provided in the kit is sufficient for directly lysing cells in 24-, 48- or 96-well culture plates. If 6- well or 12-well plates are used, we recommend either purchasing more Lysis Buffer, or harvesting cells by scraping or trypsinization according to the procedures in B of preparing cell lysates.

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 1X LucyQ FL Lysis Buffer. 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 1X LucyQ FL Cell Lysis Buffer to ensure complete lysis.

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Note : The Firefly luciferase contained in the cell lysates is 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.

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.

B. Lysis of Cells in tube

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

2. Add 50-100µL of 1X LucyQ FL Cell Lysis Buffer 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 luciferase 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 1X LucyQ FL Cell Lysis Buffer is good for the assay in normal transfected cells. If the cells have lower transfection efficiency or the promoter is very weak, you may increase the cell numbers. This Lysis Buffer is optimized for compatible with the following firefly luciferase detection assays. If other cell lysis buffers are used, the signal strength of the luciferases could be affected.

Luciferase Assay Procedure

A. Protocol for Manual Luminometers

1. Dispense 100µl of Firefly Luciferase Working solution(FLWS) into luminometer tubes, one tube per sample or 96-well white (opaque) or black plate.

2. Program the luminometer to perform a 2-second measurement delay followed by a 10-second measurement read for luciferase activity. The read time may be shortened if sufficient light is produced.

Note : When using shorter assay times, validate the luminometer over that time period to ensure that readings are taken at a flat portion of the signal curve.

3. Add 20µl of cell lysate to a luminometer tube containing the Firefly Luciferase Working Solution(FLWS). Mix by pipetting 2–3 times or vortex briefly.

4. Place the tube in the luminometer and initiate reading.

5. If the luminometer is not connected to a printer or computer, record the reading.

B. Protocol for Plate-Reading Luminometers

1. Program the luminometer for the appropriate delay and measurement times.

2. Place the plate, containing 20µl of cell lysate per well, into the luminometer with injector. The injector adds 100µl of Firefly Luciferase Working Solution(FLWS) per well, then the well is read immediately. The plate is advanced to the next well for a repeat of the inject-then-read process.

3. Measure the light produced for a period of 10 seconds. The light intensity of the reaction is nearly constant for about 1 minute and then decays slowly, with a half-life of approximately 10 minutes. The typical delay time is 2 seconds, and the typical read time is 10 seconds. The assay time may be shortened significantly to decrease the total read time if sufficient light is produced. For example, the total read time for all samples in a 96-well plate can be less than 5 minutes.

C. Protocol for Single-Tube Luminometers with Injectors

1. Prime the luminometer injector at least three times with Firefly Luciferase Working Solution(FLWS) or as recommended in the owner's manual.

2. Dispense 20µl of cell lysate or test sample into a luminometer tube.

3. Program the luminometer to perform a 2-second measurement delay followed by a 10-second measurement read for luciferase activity. The read time may be decreased if sufficient light is produced.

4. Place the tube in the luminometer and initiate reading by injecting 100µl of Firefly Luciferase Working Solution(FLWS) into the tube.

5. If the luminometer is not connected to a printer or computer, record the reading.