Luc-Pair™ Duo-Luciferase HS Assay Kit

For luciferase assays

Cat. No. LF004 (100 reactions)

Cat. No. LF005 (300 reactions)

Cat. No. LF006 (1000 reactions)

User Manual

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USER MANUAL

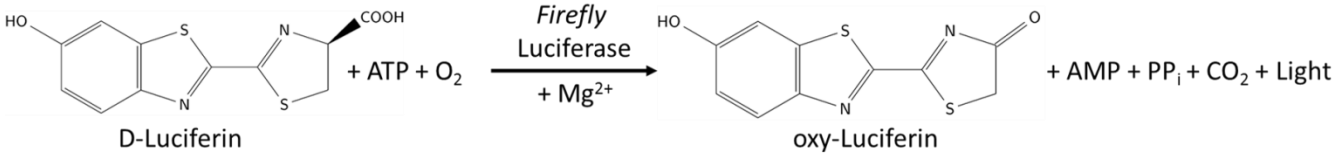
Luc-Pair™ Duo-Luciferase HS Assay Kit

I. Introduction and Principles

The study of transcriptional regulation using reporter gene expression is common and necessary in cell biology research and pharmaceutical discovery. Luciferase is the most widely used genetic reporter for gene expression studies due to several advantages, including:

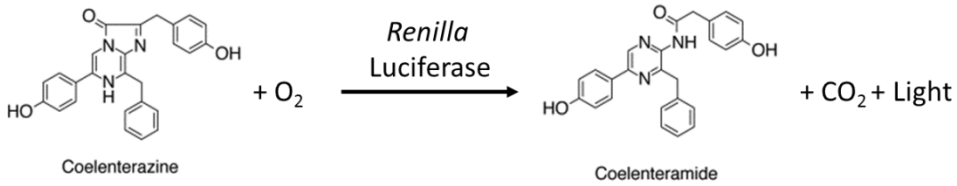
1) high sensitivity in a large dynamic range
2) natural absence from mammalian cells
3) consistent reproducibility
4) cost effectiveness
5) simple assay format

Firefly and *Renilla* luciferases have been widely used as co-reporters for normalization studies because both assays 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. It is a cytoplasmic enzyme with a molecular weight of 61 kDa and catalyzes the following reaction:



The intensity of light emission is proportional to the amount of luciferase and is measured using a luminometer or multi-function micro-plate reader.

Renilla (Renilla reniformis) luciferase is a 36 kDa monomeric protein, which requires no post-translational processing. Therefore, it can function as a real-time transcription reporter. It catalyzes the following reaction:



Using this assay system allows one to monitor the transcriptional activation of *cis*-elements in proximity to the gene of interest. However, it has been more difficult to measure the transcriptional repression via 3' UTR regulation of genes since the enzyme-substrate activity window is relatively small. Longer stability of the enzyme-substrate complex allows greater flexibility in monitoring true repressive events. Further, biological variation and stochastic events may add noise, thereby reducing the differences in observed luciferase activity. Thus, normalizing the expression of an experimental reporter to the expression of an independent control reporter can help differentiate between true signal and nonspecific cellular responses. Normalization is also needed for adjusting differences in transfection efficiencies and cell viability.

GeneCopoeia has leveraged the differences in Firefly and *Renilla* enzyme structures and substrates to optimize and develop a convenient system for measuring two luciferase activities in succession. The assay measures the activities of Firefly and *Renilla* luciferases sequentially from a single sample. The Firefly luciferase luminescence is produced by one reagent, while a second reagent simultaneously quenches the Firefly luciferase and produces *Renilla* luciferase luminescence.

The GeneCopoeia Luc-Pair™ Duo-Luciferase HS Assay Kit development team incorporated several features into the reagents to enhance product performance and convenience, including the following:

- **High sensitivity.** The reagents have been developed so that the signals for Firefly and *Renilla* luciferases exhibit greatest sensitivity (Figure 1).
- **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 and substrate 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.

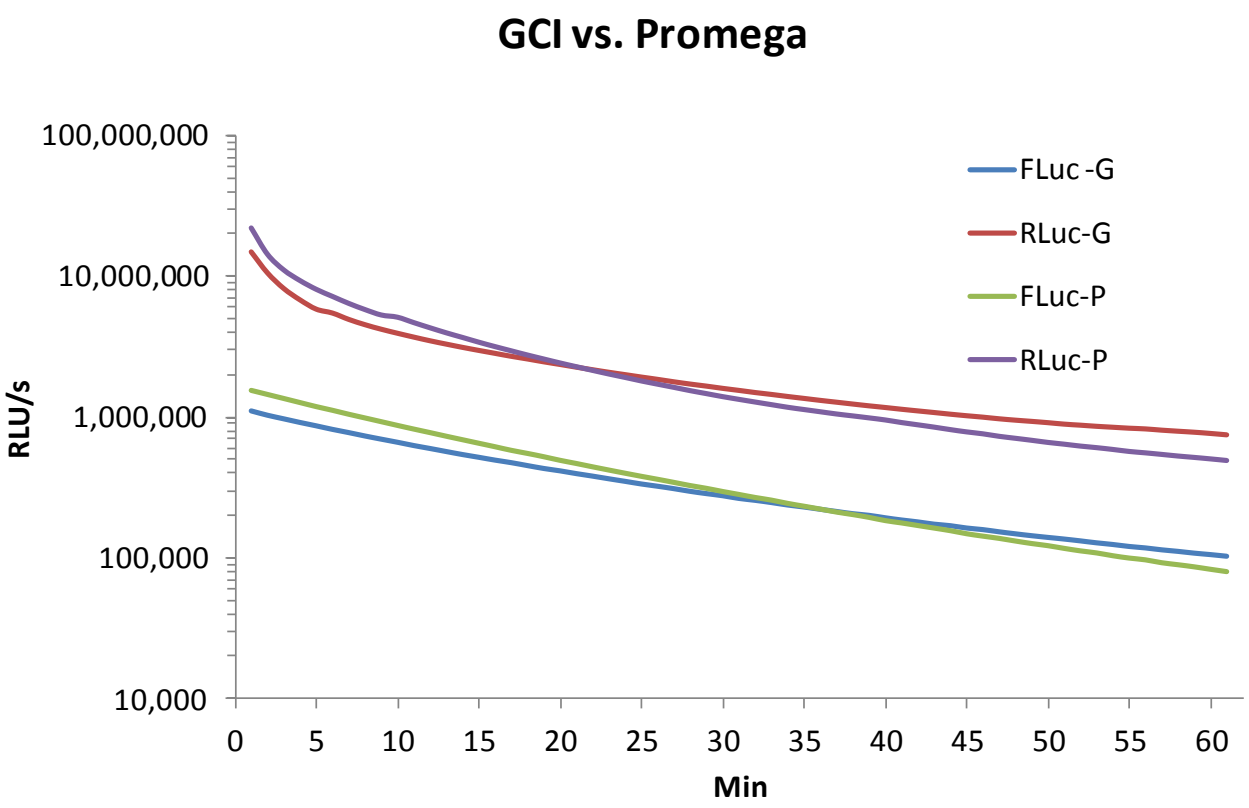


Figure 1. Activity of Firefly luciferase and *Renilla* luciferase signals using GeneCopoeia (GCI) Luc-Pair Duo-Luciferase HS Assay Kit. HEK 293 cells were transfected with Promega pGL4.13/pGL4.75 reporter vectors for 48 hours. The FLuc (FLuc-G) and RLuc (RLuc-G) activity was measured as described in the procedure. The Promega's dual-luciferase assay kit was used (FLuc-P and RLuc-P) in comparison.

II. Contents and Storage

Cat. Nos. LF004, LF005 and LF006

Contents	**Quantity** 100 reactions 300 reactions 1000 reactions	**Shipping temperature**	**Storage temperature**
Luc-Lysis II Buffer (10×) Cell Lysis buffer	1.0 mL 1.0 mL×3 10 mL	Ice pack	–20°C Stable for at least 6 months
Luc-H Buffer I (5×) Firefly luciferase buffer	1.0 mL×2 1.0 mL×6 10 mL×2	Ice pack	–20°C Stable for at least 6 months
Luc-H Sub I (100×) Firefly luciferase substrate	100 µL 100 µL×3 500 µL×2	Ice pack	–20°C Stable for at least 6 months
Luc-H Buffer II (5×) *Renilla* luciferase buffer	1.0 mL×2 1.0 mL×6 10 mL×2	Ice pack	–20°C Stable for at least 6 months
Luc-H Sub II (100×) *Renilla* luciferase substrate	100 µL 100 µL×3 500 µL×2	Ice pack	–20°C Stable for at least 6 months

III. Preparation of Cell Lysates Using Lysis Buffer

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

2. Dispense into each culture well the minimum volume of 1×LucLysis II Buffer required to completely cover the cell monolayer. The recommended volumes of 1×LucLysis II Buffer to add per well are as follows:

Culture Plate	1×LucLysis II Buffer (μL)
6-well	500
12-well	250
24-well	100
48-well	65
96-well	20

Note: The LucLysis II Buffer 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 LucLysis II Buffer (Cat. No. LF004) or harvesting cells by scraping or trypsinization according to the procedures in **III-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 1×Lysis Buffer. Rock the culture plates at room temperature for 10-15 minutes.

Note1: 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. Overgrown cells are more resistant to complete lysis, and typically require an increased volume of LucLysis II Buffer to ensure complete lysis.

Note2: 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 3 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 tubes.

5. 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 5minutes, and completely remove the rinse solution.

6. Add 50-100uL of 1×LucLysis II Buffer to make 2×10^3 cells/μL, 1 or 2 freeze-thaw cycles to accomplish complete lysis of cells.

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 3 freeze-thaw cycles may result in gradual loss of luciferase reporter enzyme activities. 2×10^3 cells/μL in 1×LucLysis II Buffer is

good for the assay in normal transfected cells. If the cells have very lower transfection efficiency or the promoter is very weak, you may increase the cell numbers.

7. Proceed to Luciferase assay.

IV. Preparation of FLuc and RLuc Assay Working Solution

Note1. Luc-H Buffers I and II are stable at –20°C for at least 6 months. Freezing and thawing the reagents3-4 cycles won't affect the activity of the luciferases. Aliquotting is recommended if more freeze-thaw cycles are required.

Note2. Working Solutions (Buffers contain 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.

1. Thaw Luc-H Buffer I (5×) and Luc-H Buffer II (5×) thoroughly at room temperature, inverting the tube several times and then vortexing for 3-5 seconds.

   Note: Some pellets may appear in the Luc-H Buffer II (5×) 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 1×Luc-H Buffer I and 1×Luc-H Buffer II. 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 1×Luc-H Buffer I and 1×Luc-H Buffer II. By diluting 0.2mL of each 5×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 Luc-H Sub I and II (100×) to 1 mL of 1×Luc-H Buffer I and 1×Luc-H Buffer II, 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.

V. 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 from step IV-4 (100 µL per well or tube) 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 descript in the manual of the devises

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 from Step IV-4 (100 µL per well or tube) to the plates or tubes from Step V-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 descript in the manual of the devises

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.

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. These normalized ratios will remain essentially constant (±10%) for samples in a plate measured during the 1-hour measurement window.

VII. Limited Use License and Warranty

Limited Use License

Following terms and conditions apply to use of the Luc-Pair™ Duo-Luciferase Assay Kit 2.0 (the Product). If the terms and conditions are not acceptable, the Product in its entirety must be returned to GeneCopoeia within 5 calendar days. A limited End-User license is granted to the purchaser of the Product. The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged or modified for resale, or used to manufacture commercial products or deliver information obtained in service without prior written consent from GeneCopoeia. This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research. Use of any part of the Product constitutes acceptance of the above terms.

Limited Warranty

GeneCopoeia warrants that the Product meets the specifications described in the accompanying Product Datasheet. If it is proven to the satisfaction of GeneCopoeia that the Product fails to meet these specifications, GeneCopoeia will replace the Product. In the event a replacement cannot be provided, GeneCopoeia will provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to GeneCopoeia within 30 days of receipt of the Product. GeneCopoeia's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. GeneCopoeia's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. GeneCopoeia does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

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