GeneCopoeia™
Expressway to Discovery

Luc-Pair™ Duo-Luciferase HT Assay Kit

For Firefly and *Renilla* luciferase

Cat. No. LF013 (10ml)
Cat. No. LF014 (30ml)
Cat. No. LF015 (100ml)

User Manual

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USER MANUAL

Luc-Pair™ Duo-Luciferase HT Assay Kit

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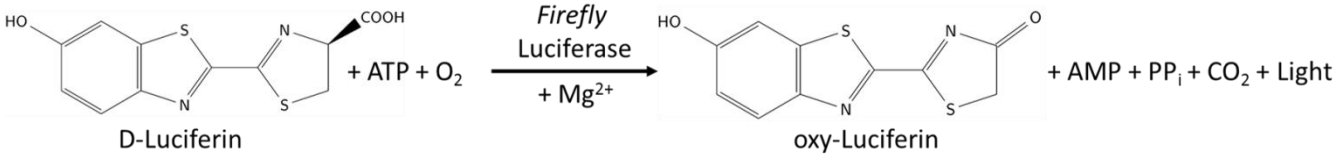
I. Introduction and Principles

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

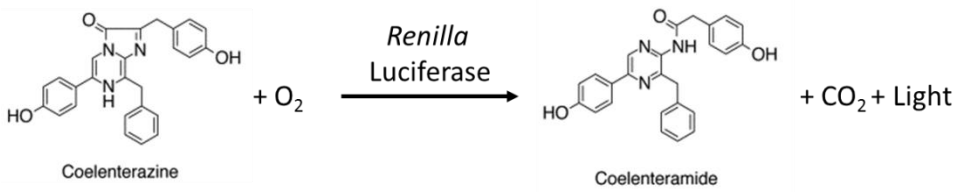
Firefly and *Renilla* luciferases have been widely used as co-reporters for normalization studies because both assays are quick, easy and sensitive. Firefly and *Renilla* luciferases are ideal co-reporters because they have distinct evolutionary origins and very different enzyme structures and substrates. 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:



D-Luciferin oxy-Luciferin

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:



GeneCopoeia has developed a convenient system for measuring two luciferase activities from a single sample in succession. The Luc-Pair™ Duo-Luciferase HT Assay reagents can be added directly to cells in growth medium without washing or preconditioning. It has been optimized for use with the following types of media containing 0–10% serum: DMEM, RPMI1640, EMEM, IMDM, McCoy's-5A, F-12K, MEBM, ACL-4, L15, Cho-S-SFMII, NCTC109. 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 HT Assay Kit development team incorporated several features into the reagents to enhance product performance and convenience, including the following:

- **Enhanced stability.** The reagents have been developed so that the signals for firefly and *Renilla* luciferases exhibit greater stability and have a half-life of approximately 2 hours. (Figure 1). An idea system for high through-put assays
- **Convenient.** Directly lyse cells in culture medium and measure luciferase activities simultaneously. It has been optimized for use with variant of media (Figure 2)
- **Versatility.** The system has been designed for assays with many different eukaryotic (adherent or suspended) cells using micro-plates luminescence readers.
- **Low background.** The system produces very limited background luminescence. No subtraction is required from readings.
- **Simplicity.** *Renilla* luciferase buffer contains the quenchers for Firefly luciferase activity (Figure 3). 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.

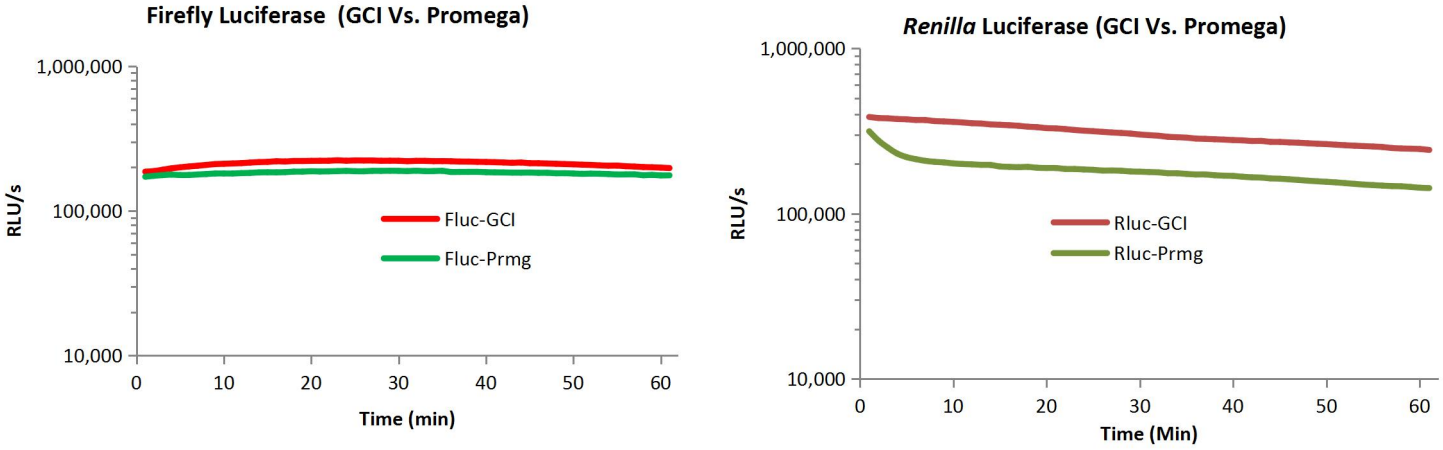


Figure 1. Activities of Firefly and *Renilla* luciferase signals using the GeneCopoeia (GCI) Luc-Pair Duo-Luciferase HT Assay Kit. HEK 293 cells were transfected with Promega pGL4.13/pGL4.75 reporter vectors for 48 hours. FLuc and RLuc activities were measured as described in the procedure. Promega's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

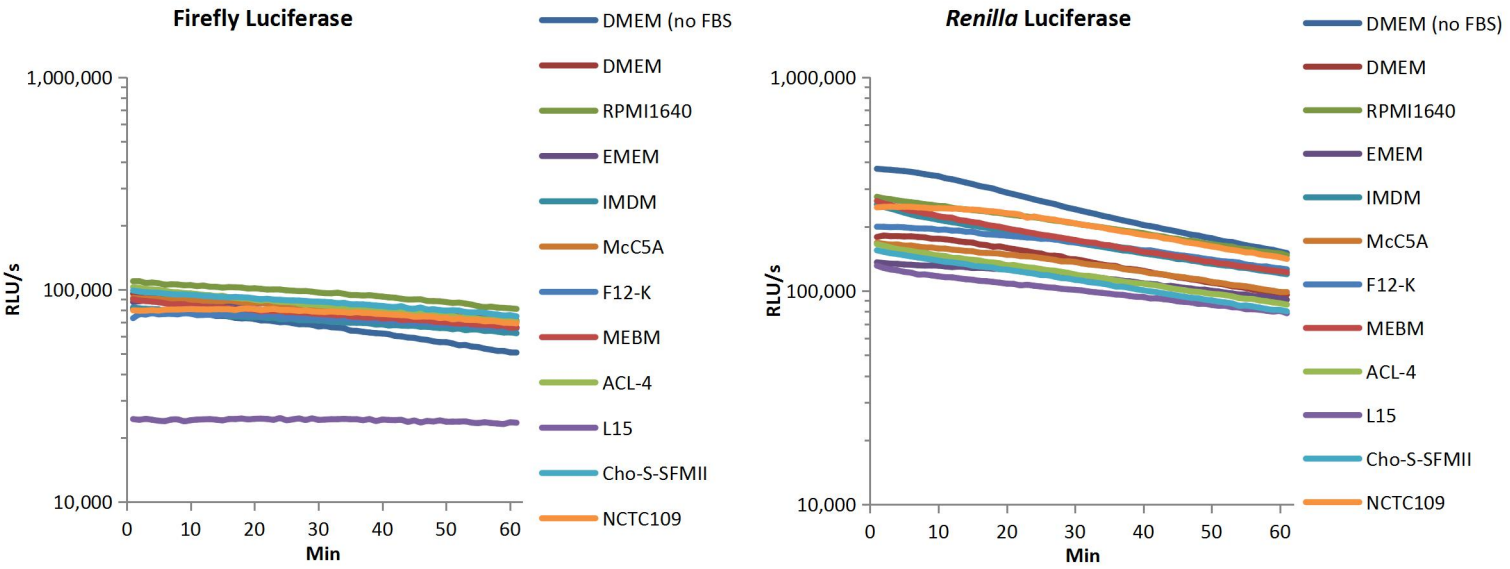


Figure 2. Firefly and Renilla luciferase activity mesured in various media. HEK 293 cells were transfected with Promega pGL4.13/pGL4.75 reporter vectors for 48 hours. 5x10^4 transfected cells were suspended in DMEM without serum or the following types of media containing 10% serum: DMEM, RPMI1640, EMEM, IMDM, McCoy's5A, F−12K, MEBM, ACL-4, L15, Cho-S-SFMII, NCTC109. Firefly and *Renilla* luciferase activates were measured as described in the procedure.

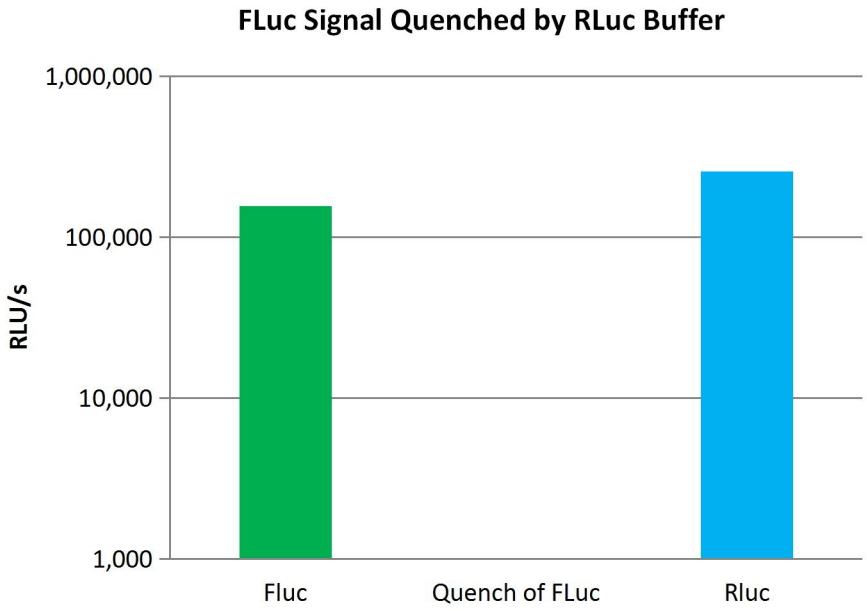


Figure 3. Firefly luciferase activity is quenched with the addition of Luc-HT Buffer II. HEK 293 cells were transfected with Promega pGL4.13/pGL4.75 reporter vectors for 48 hours. Firefly luciferase activity was measured as described in the procedure. Next, 1×Luc-HT Buffer II (without or with substrate) was added to the wells, followed by count reading in a luminometer. About 99.9% of firefly luciferase activity was quenched (middle column).

II. Contents and Storage

Contents Cat. Nos. LF013 Cat. Nos. LF014 Cat. Nos. LF015	**Quantity** 10ml 30ml 100ml	**Shipping temperature**	**Storage temperature**
Luc-HT 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-HT 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-HT 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-HT 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 Cells cultured in Multi-well Plates

Use the plates that are compatible with the type of luminometer for cell culture. For both adherent and suspension cells, don't allow the cells to overgrow at the desired time of the assay. Use an appropriate volume of growth medium for culturing cells in the wells. Typically, 75µl of medium is used for 96-well plates, and 20µl of medium is used for 384-well plates.

IV. Preparation of FLuc and RLuc Assay Working Solution

Note1. Luc-HT Buffers I and II are stable at –20°C for at least 6 months. Freezing and thawing the reagents 5-6 cycles will not affect the activity of the luciferases. Aliquotting is recommended if more freeze-thaw cycles are required.

Note2. 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 measurements.

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

   Note1: The Luc-HT Buffers I (5×) might turn turbid after thawing. This will not affect the assays. Just mix well by vortexing before pipetting .

   Note2: The Luc-HT Buffers II (5×) might have some pellets appear after thawing. It is important to completely dissolve the pellets before use. Incubation at 37°C for 5-10 minutes and more vortexing will be necessary to fully re-dissolve the pellets.

2. Dilute 1:5 Luc-HT Buffer I (5×) and Luc-HT Buffer II (5×) in distilled water to make 1×Luc-HT Buffer I and 1×Luc-HT Buffer II. Depending on the volume of medium in the cultured cells, prepare equal volume of each Buffer for each reaction/well. For 96-well plates, typically 75µl of reagent is needed for cells grown in 75µl of medium. For 384-well plates, typically 20µl of reagent is needed for cells grown in 20µl of medium), Duplicates or triplicates for each sample are recommended.

   Example: If you are testing 30 samples with duplications (total 60 reactions) in 96-well plates, prepare 5 mL of each 1×Luc-HT Buffer I and 1×Luc-HT Buffer II by diluting 1.0 ml of each 5X Buffers with 4 ml of ddH2O respectively. Preparing some extra will be helpful to avoid buffer shortage caused by the pipetting error.

3. Prepare the **FLuc and RLuc Assay Working Solution** by diluting Luc-HT Sub I and II (100×) 1:100 into an appropriate volume of 1×Luc-HT Buffer I and 1×Luc-HT Buffer II respectively. Mix well by inverting the tube several times.

   Example: For preparing 5 mL of each **FLuc and RLuc Assay Working Solutions,** add 50 µL of Luc-HT Sub I to 5 mL of 1× Luc-HT Buffer I and 50 µL of Luc-HT Sub II (100×) to 5 mL of 1×Luc-HT 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: Assay Working Solutions (Buffers contain Substrates) are stable at room temperature for 1-2 hours. Prepare only the required amount of Assay Working Solution before use. The **RLuc Assay Working Solution** will be used after reading the FLuc assay

V. Assay Procedures

5. Set up the luminometer. Follow the manual associated with your plate reader. Set the measurement for 1-2 seconds of integration.
6. Remove multiwell plates containing cells from the incubator. Make certain that the plates are compatible with the type of luminometer being used.
7. Add a volume of **FLuc Assay Working Solution** equal to the culture medium volume to each well and mix. For 96-well plates, typically 75µl of reagent is added to cells grown in 75µl of medium. For 384-well plates, typically 20µl of reagent is added to cells grown in 20µl of medium.

   Note: Auto-Injector is not recommended for this kit.

8. Place the culture plates on a rocking platform or orbital shaker with gentle rocking/shaking for at least 10 minutes,
9. Proceed with the measurement. Save the reading if the luminometer reader does not automatically record the readings.
10. Remove the plates from the luminometer .
11. Add a volume of **RLuc Assay Working Solution** equal to the original culture medium volume to each well and mix. As noted in Step 7, this volume is typically 75µl for 96-well plates and 20µl for 384-well plates.
12. Wait for 2-5 min, then proceed with the measurement.
13. Record the reading if the luminometer reader does not automatically save the readings.
14. Remove the plates.
15. 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. This kit is not designed for single luciferase detection. If using for detection of 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.

VII. Limited Use License and Warranty

Limited Use License

The following terms and conditions apply to use of the Luc-Pair™ Duo-Luciferase HT Assay Kit (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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