Luc-Pair™ Firefly Luciferase HT Assay Kit

For Firefly luciferase assays

Cat. No. LF016 (10ml)
Cat. No. LF017 (30ml)
Cat. No. LF018 (100ml)

User Manual

GeneCopoeia, Inc.
9620 Medical Center Drive, #101
Rockville, MD 20850
USA
301-762-0888
866-360-9531
inquiry@genecopoeia.com
www.genecopoeia.com

USER MANUAL

Luc-Pair™ Firefly Luciferase HT Assay Kit

I. Introduction and Principles

II. Contents and Storage

III. Preparation of Cells cultured in Multi-well Plates

IV. Preparation of FLuc Assay Working Solution

V. Assay Procedures

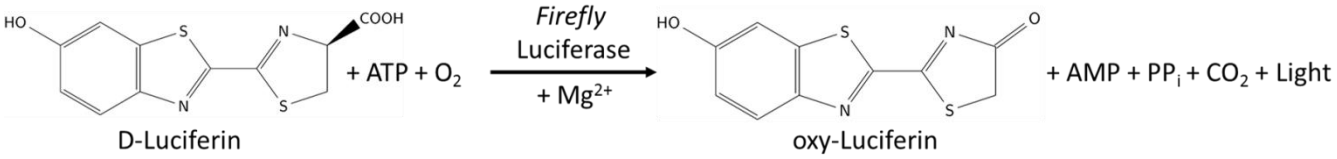
VI. Limited Use License and Warranty

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 (*Photinus pyralis*) luciferase has been widely used as reporter because the assay is quick, easy and sensitive. Firefly 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.

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 developed a convenient system for measuring Firefly luciferase activities from a single sample. The Luc-Pair™ Firefly 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, this reagent induces cell lysis and exhibit great stable signals for firefly luciferases.

The GeneCopoeia Luc-Pair™ Firefly 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 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.**
- **Reproducibility.** This system is designed to yield reliable, linear results for a concentration range over several orders of magnitude.

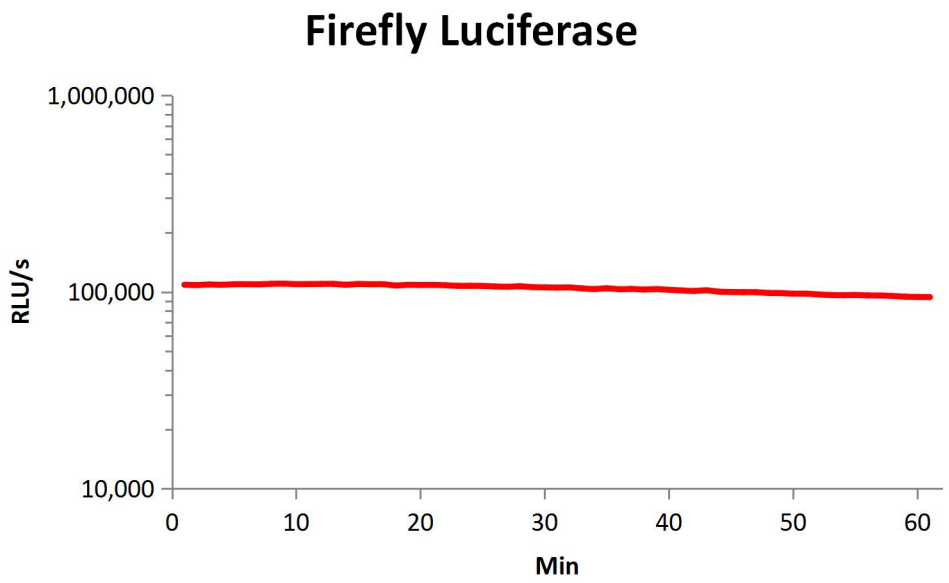


Figure 1. Activity of Firefly luciferase. HEK 293 cells were transfected with Promega pGL4.13 reporter vectors for 48 hours. The Fluc activity was measured using the Luc-Pair™ Firefly Luciferase HT Assay Kit as described in the procedure.

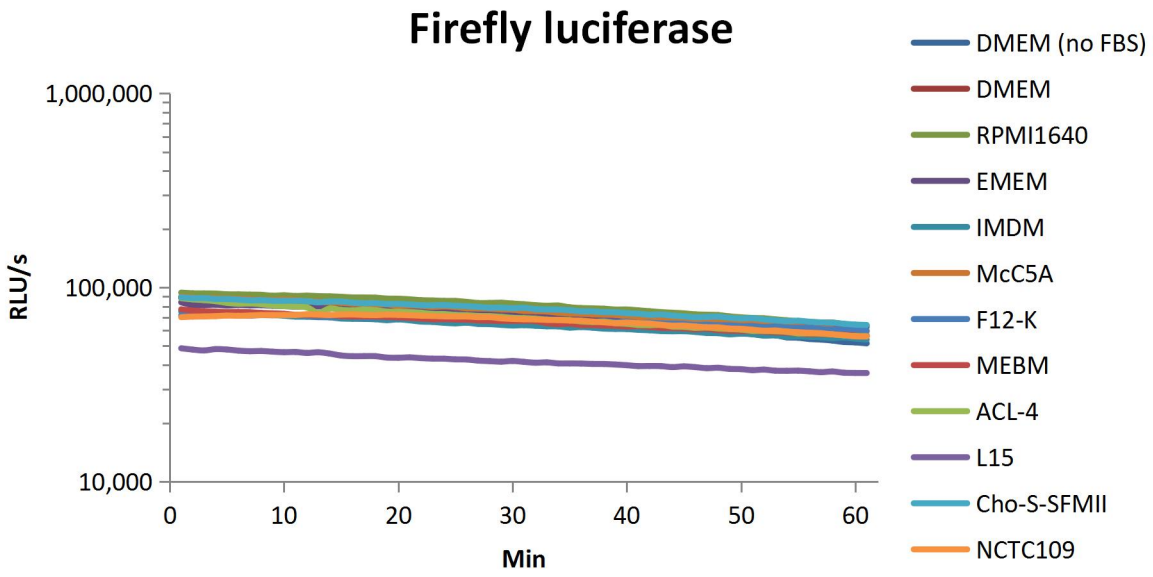


Figure 2. Firefly luciferase activities in various media. HEK 293 cells were transfected with Promega pGL4.13/pGL4.75 reporter vectors for 48 hours. 5x104 transfectd cells were suspended in DMEM without serum or the following types of media containing 10% serum: DMEM, RPMI1640, EMEM, IMDM, McCoy's5A, F-12K, MEBM, ACL-4, L15, Cho-S-SFMII, NCTC109. The Firefly luciferase activities were measured using the Luc-Pair™ Firefly Luciferase HT Assay Kit as described in the procedure.

II. Contents and Storage

Contents Cat. Nos. LF016 Cat. Nos. LF017 Cat. Nos. LF018	**Quantity** 10ml 30ml 100ml	**Shipping temperature**	**Storage temperature**
FLuc-HT Buffer (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
FLuc-HT Sub (100×) Firefly 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 Assay Working Solution

Note1. FLuc-HT Buffer is 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 FLuc-HT Buffer (5×) thoroughly at room temperature, inverting the tube several times, and then vortex for 3-5 seconds.

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

2. Dilute 1:5 FLuc-HT Buffer (5×) in distilled water to make 1×FLuc-HT Buffer. 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 1×FLuc-HT Buffer 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 Assay Working Solution** by diluting FLuc-HT Sub (100×) 1:100 into an appropriate volume of 1×FLuc-HT Buffer. Mix well by inverting the tube several times.

   Example: For preparing 5 mL of each **FLuc Assay Working Solutions,** add 50 µL of FLuc-HT Sub(100×) to 5 mL of 1× FLuc-HT 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: 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.

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 .

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.

VII. Limited Use License and Warranty

Limited Use License

The following terms and conditions apply to use of the Luc-Pair™ Firely 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.

GeneCopoeia is committed to providing our customers with high-quality products. If you should have any questions or concerns about any GeneCopoeia products, please contact us at 301-762-0888.