

## How To Choose a GeneCopoeia Luciferase System

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### Introduction

Luciferase reporter systems are invaluable tools for several applications, including regulation of gene expression and high-throughput compound screening (reviewed in Thorne, et al., 2010). GeneCopoeia has developed several luciferase reporter systems, offering a range of options depending on the situation. In this Technical Note, we discuss the differences between GeneCopoeia luciferase reporter systems, and how to choose the GeneCopoeia luciferase system that best benefits your research.

### Applications for luciferase reporters

For some applications calling for reporters that generate light, luciferases offer great benefits compared with fluorescent reporters, like green fluorescent protein (GFP) or fluorescein. First, fluorescent reporters can have high background, resulting from overlap of the excitation and emission wavelengths as well as natural background fluorescence of cells. Second, fluorescent reporters suffer from photobleaching from excess excitation. This limits the window of time that the reporter is available for detection. In contrast, luciferases emit light resulting from oxidation of a substrate, and are not found in most organisms used for study. Therefore, luciferases provide high signal-to-noise ratios and are not destroyed by the experiment.

Some popular applications for luciferase reporters include:

- **Monitoring regulation of gene expression.** This is often achieved by fusing a luciferase reporter gene to a gene promoter or other 5' regulatory element, or to a microRNA (miRNA) 3' UTR binding site. GeneCopoeia's GLuc-ON™ promoter-reporter clones and miTarget™ 3'UTR target clones can be used in these applications.
- **Monitoring biological responses of cells to compound treatments.** Typically accomplished by expressing a luciferase reporter in cells fused to a gene promoter or other 5' regulatory element, and measuring luciferase activity after treatment of the cells with one or more chemical compounds. GeneCopoeia's GLuc-ON™ Transcriptional Response Element (TRE) clones and stable cell lines can be used for these applications.
- **In vivo cancer tracking.** Luciferases are often stably integrated into the genomes of cancer cell lines, which can then be injected into mice or other animal models. The luciferase is used to monitor the response of the cancer cells to various experimental manipulations, such as gene mutations or drug treatments. GeneCopoeia's labeled cancer cell lines can be used for these applications.

### GeneCopoeia luciferase assay systems

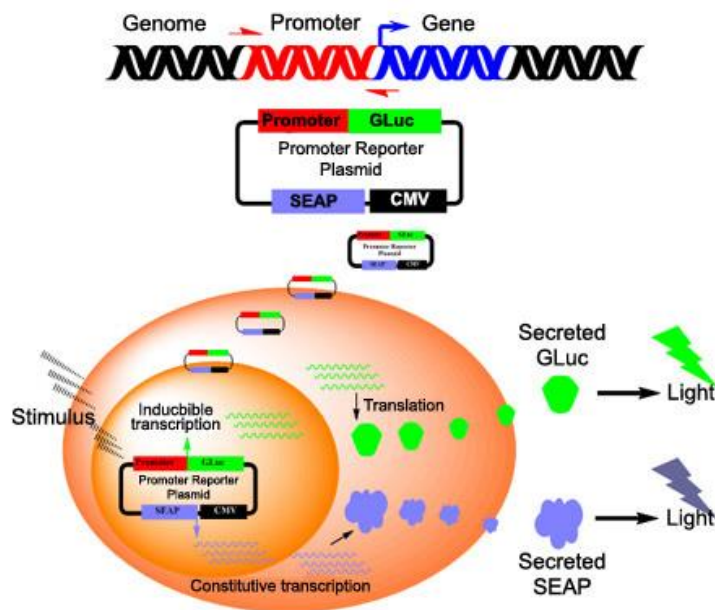
GeneCopoeia offers an extensive array of luciferase assay products that rely on two reporter systems: The first is a secreted reporter system, comprised of *Gaussia* luciferase and secreted alkaline phosphatase

(SEAP). The second employs the more traditional Firefly and *Renilla* luciferases (FLuc and RLuc, respectively).

### Secreted reporters

Some luciferases found in nature are secreted proteins. One such luciferase comes from the copepod *Gaussia princeps* (GLuc; Tannous, et al., 2005). Because GLuc is secreted, its activity is assayed in the cell culture medium, enabling measurement of reporter activity in live cells. For time course studies, that means that the same sample can be assayed multiple times.

GeneCopoeia scientists have developed a powerful and highly versatile set of tools that take advantage of the secreted properties of GLuc. The Secrete-Pair™ Dual Luminescence Assay System measures the activities of both GLuc and SEAP in the cell culture medium. SEAP is not a luciferase, but its chemiluminescent signal serves as a control to normalize the GLuc levels that might vary based on cell transfection efficiency or viability (Figure 1).



**Figure 1.** GeneCopoeia's Secrete-Pair™ Dual Luminescence Assay System. Promoter activity analysis. Top: A presumed gene promoter (region of the chromosome 5' of a gene) is inserted upstream of the *Gaussia princeps* luciferase (GLuc) ORF. The plasmid backbone also contains the secreted alkaline phosphatase (SEAP) gene under control of a constitutive promoter (CMV). Cells are transfected with the clone. At the desired time, an aliquot of cell culture media is assayed for light production by addition of the appropriate, substrate-containing reagents from the kit. The same sample can be assayed again multiple times without cell lysis.

### Traditional luciferase reporters

Traditional luciferase reporter systems use the Firefly (*Photinus pyralis*) luciferase, and often include a second luciferase, from the sea pansy *Renilla reniformis*. The *Renilla* luciferase is usually used as a normalization control (Grentzmann, et al., 1998).

While Firefly and *Renilla* luciferases have for many years provided researchers with an efficient means to measure reporter activity with superb signal to noise ratio, they are not ideal for live cell studies. FLuc and RLuc are intracellular, so cells must be lysed to release the enzyme, add substrate, and measure activity. This can add two potential variables to experiments that can weaken reproducibility: 1) Lysis efficiencies

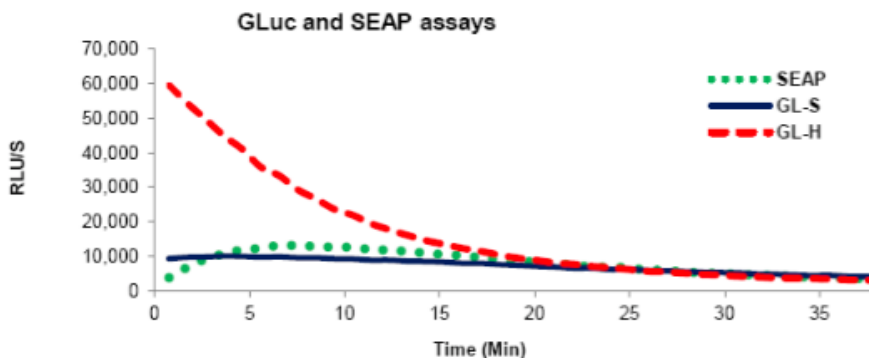
can vary among multiple samples; and 2) For time course experiments, multiple samples for each condition must be analyzed. Therefore, for live cell studies, GeneCopoeia recommends using the secreted GLuc and SEAP reporters.

GeneCopoeia has developed several different versions of both secreted and traditional luciferase kits, depending on the application. Each version differs from the other either in the luciferase buffer formulation, which affects the stability and strength of the luciferase signal, or, in the case of FLuc and RLuc, in the lysis buffer. These kits are well-suited for many different eukaryotic cell types (e.g. mammalian, invertebrates, etc.) using micro-plate or single-tube luminometers.

### Applications requiring high sensitivity

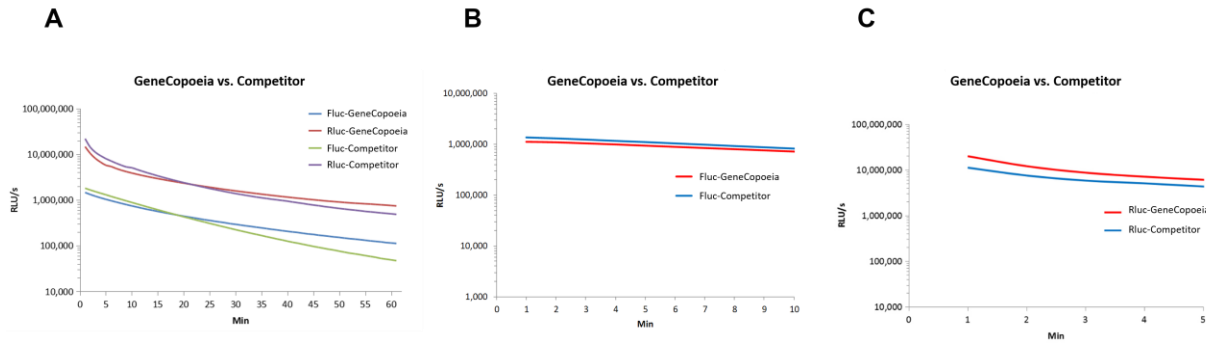
The sensitivity of the luciferase signal can be modulated by altering the buffer composition. In some situations, the highest possible sensitivity is required, e.g. in cases where source material is limiting. Typically, though, signals with high sensitivity are unstable, and have short half-lives, so these luciferase systems are best suited for use with either a small number of samples, or in luminometers that have microinjectors, which inject the assay reagents into wells simultaneously and at a much faster rate than what can be achieved through manual pipetting.

For applications requiring the greatest signal sensitivity, GeneCopoeia recommends the Secrete-Pair™ system, based on GLuc, which provides greater signal intensity than that of both FLuc or RLuc (Tannous, et al., 2005). Two buffer conditions are provided in the kits for GLuc assays depending on the application. When higher signal intensities are needed, buffer GL-H is used. On the other hand, buffer GL-S is used for stabilized activity by overcoming the quick decay of the GLuc signal instead. However, stabilized GLuc activity is associated with a lower initial signal intensity (Figure 2).



**Figure 2.** GLuc and SEAP assays. Cell culture medium was collected from cells transfected with wild-type (wt) GLuc-SEAP dual-reporter clone. 10  $\mu$ l of the medium was used in each assay. At the beginning, the GLuc activity in Buffer GL- H is about 4-6 times higher than that in Buffer GL-S, then quickly decays. The GLuc activity in Buffer GL-S, however, is much more stable.

GeneCopoeia also offers high-sensitivity versions of its FLuc and RLuc systems: The Luc-Pair™ HS (High Sensitivity) assays kits, which are available in either a duo-luciferase configuration (FLuc + RLuc), or for FLuc or RLuc alone. Similar to using Buffer-H with Secrete-Pair™, the Luc-Pair™ HS buffers are formulated to provide the highest levels of sensitivity (Figure 3).

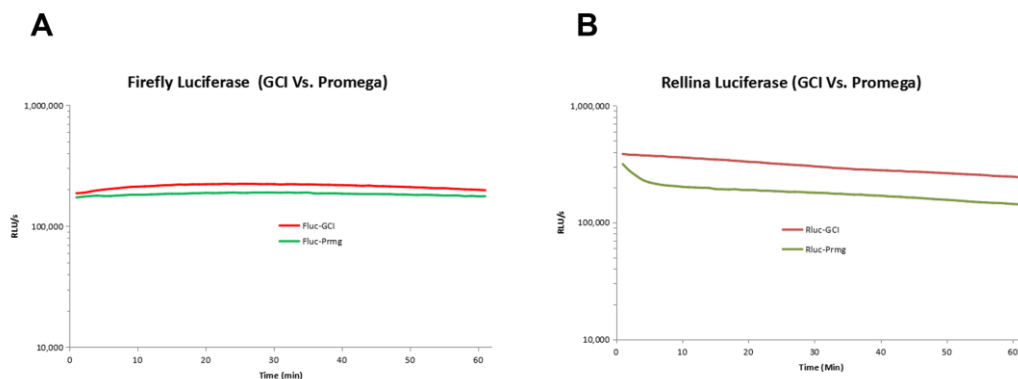


**Figure 3.** Performance of GeneCopoeia’s Luc-Pair™ HS Assay kits vs. those from a leading competitor. A. HEK293 cells were transfected with pGL4.13 and pGL4.75 reporter vectors (Promega) for 48 hours. FLuc (FLuc-GeneCopoeia) and RLuc (RLuc-GeneCopoeia) activities were measured as described in the protocol. A Competitor’s dual-luciferase assay kit was used (FLuc-Competitor and RLuc-Competitor) in comparison. B. HEK293 cells were transfected with pGL4.13 reporter vector (Promega) for 48 hours. FLuc (FLuc-GeneCopoeia) activity was measured as described in the protocol. A Competitor’s Luciferase Assay System was used (FLuc-Competitor) in comparison. C. HEK293 cells were transfected with pGL4.75 reporter vector (Promega) for 48 hours. RLuc (RLuc-GeneCopoeia) activity was measured as described in the protocol. A Competitor’s Luciferase Assay System was used (RLuc-Competitor) in comparison.

### Applications requiring greater signal stability

In some situations, a longer luciferase signal half-life is needed. For example, if you are working with larger numbers of samples, and you are not using a sample injector, the luciferase signal intensities from high-sensitivity formulations will decay too quickly for you to get consistent readings between samples. In these cases, GeneCopoeia recommends using the Secrete-Pair™ Dual luminescence assay kits with Buffer GL-S (Figure 2), which provides a GLuc signal with a half-life of more than 1 hour.

In addition, GeneCopoeia has developed versions of its traditional Firefly and *Renilla* luciferase assay kits that provide greater luciferase signal stability: The Luc-Pair™ Duo-Luciferase Assay Kit 2.0. The luciferase activities retain greater than 80% of their starting intensities 30 minutes after addition of the enzymatic substrates (Figure 4).

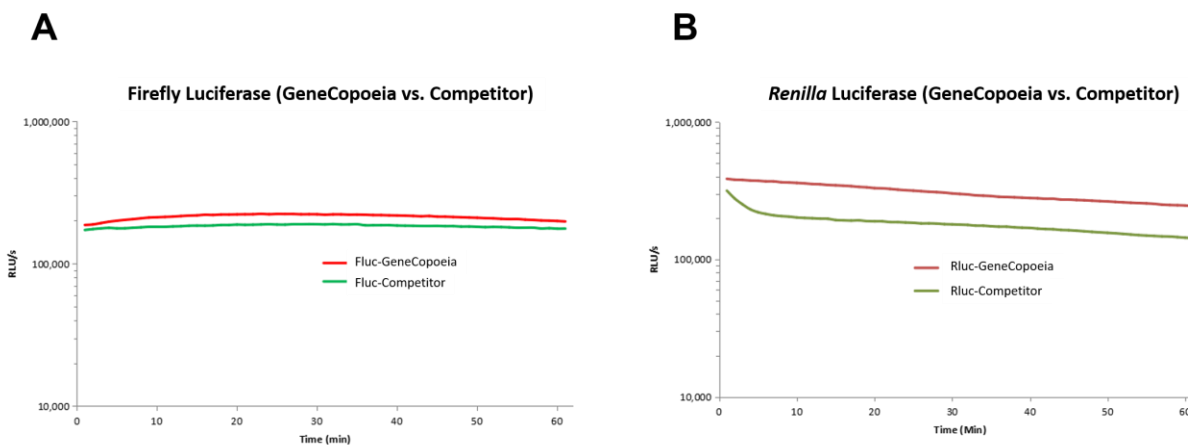


**Figure 4.** Stability of firefly luciferase and Renilla luciferase signals using GeneCopoeia (GCI) Luc-Pair™ Duo-Luciferase Assay Kit 2.0. HEK 293 cells were transfected with GeneCopoeia pEZX-MT06 miRNA reporter vector for 48 hours. A. The FLuc and RLuc activity was measured as described in the procedure. B. A Competitor’s dual-luciferase assay kit was used in comparison.

## High-throughput luciferase applications

Some applications, such as screening chemical compound libraries, require the processing and analysis of hundreds or thousands of samples at once. For this kind of high-throughput work, it is critical to minimize the number of steps carried out during sample processing. For high-throughput luciferase applications, GeneCopoeia's Secrete-Pair™ Dual Luminescence Assay kit is recommended. Because GLuc and SEAP are secreted, there is no need to lyse the samples. You simply add the reagents to the wells, place the samples into the luminometer, and measure luminescence.

For traditional FLuc and RLuc, GeneCopoeia has also developed a new series of luciferase kits intended for high-throughput experiments: The Luc-Pair™ HT Assay Kits, which are available for Firefly and *Renilla* luciferases together (Luc-Pair™ Duo-Luciferase HT Assay Kit), or for Firefly or *Renilla* luciferases alone (Luc-Pair™ Firefly Luciferase HT Assay Kit and Luc-Pair™ *Renilla* HT Assay Kit, respectively). These kits utilize a cell lysis buffer that can be added directly to the cell culture medium, so that removal of the medium and washing are unnecessary. The reagents provide high sensitivity and stability even in the presence of serum (Figure 5).



**Figure 5.** Activities of Firefly (A) and *Renilla* (B) 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. A Competitor's Dual-Glo luciferase assay kit was used (FLuc-Competitor and RLuc-Competitor) in comparison

## How do I choose the GeneCopoeia luciferase system that's right for me?

Choosing the GeneCopoeia luciferase system that best suits your needs depends on many factors. For example, for applications requiring either the highest sensitivity, greatest signal sensitivity, or live cell analysis, choose a Secrete-Pair™ kit. For compatibility with GeneCopoeia and other vendors' traditional luciferase vectors, choose one of the GeneCopoeia Luc-Pair™ Firefly and/or *Renilla* luciferase kits. Refer to Table 1 for a more detailed comparison.

	Compatibility with traditional luciferase vectors	Ultra sensitivity	Signal stability	Large sample numbers	Live cell analysis
<b>Secrete-Pair™ (Gaussia and/or SEAP)</b>		✓	✓	✓	✓
<b>Luc-Pair™ 2.0 (Firefly &amp; Renilla; for advanced stability)</b>	✓		✓		
<b>Luc-Pair™ HS (Firefly and/or Renilla; for high sensitivity)</b>	✓	✓			
<b>Luc-Pair™ HT (Firefly and/or Renilla; for high throughput)</b>	✓		✓	✓	

**Table 1.** Comparisons between different GeneCopoeia luciferase systems.

### GeneCopoeia luciferase assay kits

GeneCopoeia provides one of the most extensive selections for both secreted and traditional luciferase systems available, as shown in Table 2.

### GeneCopoeia luciferase clone products

In addition to a large variety of luciferase assay kits, GeneCopoeia manufactures and provides several categories of plasmid clones and cloning vectors that use either the Secrete-Pair™ or Luc-Pair™ assay systems, or fluorescent reporters, as described below:

- **GLuc-ON™ Promoter Reporter clones.** More than 20,000 human and more than 18,000 mouse pre-designed gene promoters, in which approximately 1.5 kb upstream to approximately 200 bp downstream of the transcription start site (TSS) has been inserted at the 5' end of GLuc. Each clone carries the constitutively-expressed SEAP gene. Promoter clones are also available with eGFP, mCherry, or tdTomato fluorescent reporters. Customers can search for their promoter clone of interest on the GeneCopoeia website. All promoter clones are transfection-ready.
- **miTarget™ microRNA target clones.** More than 18,000 human, 18,000 mouse, and 10,000 rat 3' untranslated regions (3' UTRs) cloned upstream of GLuc, designed to study miRNA-level gene regulation. Each clone carries SEAP for normalization. miRNA target clones are also available with FLuc and RLuc reporters. Customers can search for their microRNA target clone of interest by gene symbol on the GeneCopoeia website.
- **GLuc-ON™ Transcriptional Response Element (TRE) clones.** Promoter elements from genes involved in signal transduction pathways, such as NFκB and Wnt, cloned upstream of GLuc. For determining involvement of signal transduction pathway responses to environmental or chemical stimuli. Pre-made cell lines stably expressing TRE-GLuc fusions are also available.
- **GLuc cloning vectors.** For do-it-yourself cloning. Carry GLuc either without a promoter (for promoter cloning), a constitutive promoter (for 3' UTR cloning), or a minimal CMV promoter (for TRE cloning). Available with or without SEAP for normalization.

- **FLuc/RLuc cloning vectors.** For do-it-yourself cloning. Carry Firefly luciferase either without a promoter (for promoter cloning), a constitutive promoter (for 3' UTR cloning), or a minimal CMV promoter (for TRE cloning). Also express *Renilla* luciferase for normalization.

Catalog #	Product	Description
<b>Secrete-Pair™ <i>Gaussia</i> luciferase assay kits</b>		
LF031	Secrete-Pair Dual Luminescence Assay Kit (100 rxns)	Detects <i>Gaussia</i> luciferase (GLuc) and secreted alkaline phosphatase (SEAP)
LF032	Secrete-Pair Dual Luminescence Assay Kit (300 rxns)	
LF033	Secrete-Pair Dual Luminescence Assay Kit (1000 rxns)	
LF061	Secrete-Pair <i>Gaussia</i> Luciferase Assay Kit (100 rxns)	Detects <i>Gaussia</i> luciferase (GLuc)
LF062	Secrete-Pair <i>Gaussia</i> Luciferase Assay Kit (1000 rxns)	
<b>Luc-Pair™ Firefly and <i>Renilla</i> luciferase assay kits</b>		
LF001	Luc-Pair Duo-Luciferase Assay Kits 2.0 (100 rxns)	Detects Firefly luciferase (FLuc) and <i>Renilla</i> luciferase (RLuc)
LF002	Luc-Pair Duo-Luciferase Assay Kits 2.0 (300 rxns)	
LF003	Luc-Pair Duo-Luciferase Assay Kits 2.0 (1000 rxns)	
LF004	Luc-Pair™ Duo-Luciferase HS Assay Kit (100 rxns)	For high sensitivity detection of Firefly and <i>Renilla</i> luciferase activities.
LF005	Luc-Pair™ Duo-Luciferase HS Assay Kit (300 rxns)	
LF006	Luc-Pair™ Duo-Luciferase HS Assay Kit (1000 rxns)	
LF007	Luc-Pair™ Firefly Luciferase HS Assay Kit (100 rxns)	For High sensitivity for detection of Firefly luciferase activity.
LF008	Luc-Pair™ Firefly Luciferase HS Assay Kit (300 rxns)	
LF009	Luc-Pair™ Firefly Luciferase HS Assay Kit (1000 rxns)	
LF010	Luc-Pair™ <i>Renilla</i> Luciferase HS Assay Kit (100 rxns)	For High sensitivity for detection of <i>Renilla</i> luciferase activity.
LF011	Luc-Pair™ <i>Renilla</i> Luciferase HS Assay Kit (300 rxns)	
LF012	Luc-Pair™ <i>Renilla</i> Luciferase HS Assay Kit (1000 rxns)	
LF013	Luc-Pair™ Duo-Luciferase HT Assay Kit (10 ml)	For high-throughput detection of Firefly and <i>Renilla</i> luciferase activities.
LF014	Luc-Pair™ Duo-Luciferase HT Assay Kit (30 ml)	
LF015	Luc-Pair™ Duo-Luciferase HT Assay Kit (100 ml)	
LF016	Luc-Pair™ Firefly Luciferase HT Assay Kit (10 ml)	For high-throughput detection of Firefly luciferase activities.
LF017	Luc-Pair™ Firefly Luciferase HT Assay Kit (30 ml)	
LF018	Luc-Pair™ Firefly Luciferase HT Assay Kit (100 ml)	
LF019	Luc-Pair™ <i>Renilla</i> Luciferase HT Assay Kit (10 ml)	For high-throughput detection of <i>Renilla</i> luciferase activities.
LF020	Luc-Pair™ <i>Renilla</i> Luciferase HT Assay Kit (30 ml)	
LF021	Luc-Pair™ <i>Renilla</i> Luciferase HT Assay Kit (100 ml)	

**Table 2.** GeneCopoeia Secrete-Pair™ and Luc-Pair™ luciferase assay kit options.

## Conclusions

At GeneCopoeia, our scientists are continually producing high quality products for molecular and cellular biology designed to accelerate your research. For more information on our luciferase systems, visit our website, <http://www.genecopoeia.com/product/luciferase-assays/>, or call 1-866-360-9531.

## References

Gretnsman G., *et al.* (1998) A dual-luciferase reporter system for studying recoding signals. *RNA* **4**, 479.

Thorne, N., *et al.* (2010). Illuminating Insights into Firefly Luciferase and Other Bioluminescent Reporters Used in Chemical Biology. *Chemistry & Biology* **6**, 646.

Tannous B. A., *et al.* (2005). Codon-optimized Gaussia luciferase cDNA for mammalian gene expression in culture and in vivo. *Mol Ther.* **11**, 435.