Ready-To-Glow™ Secreted Luciferase System

Fast and easy detection of reporter gene activity without cell lysis

- **Easy one-step detection**
- **Allows repeated assaying over time**
- **No cell lysis required**
- **Adaptable for high-throughput screening applications**

Clontech is offering a new reporter system, the **Ready-To-Glow Secreted Luciferase System,** based on secreted *Metridia* luciferase. It combines the advantages of a live-cell assay with the sensitivity of an enzyme-based system. A "one-step" reaction allows monitoring of promoter activation by detecting the activity of the secreted reporter enzyme in the supernatant of transfected cells without the need for cell lysis.

Our system provides an efficient method for studying the interaction of transcription factors with specific promoters and response elements in the promoter region of genes, an important stage in signal transduction pathways. Several different reporter systems are currently available to monitor the activation of these promoter elements. They include substrate-independent fluorescent proteins and substrate-dependent luciferase-based assays utilizing firefly luciferase or *Renilla* luciferases. Although fluorescent protein-based reporter systems require no substrate, these systems are less sensitive than enzyme-based reporter systems. Since firefly and *Renilla* luciferases are cytosolic proteins, cell lysis must occur in order to allow access to the reporter before adding the substrate. Additionally, in time-course studies, transfected cells must be sacrificed at each interval to obtain meaningful data. Our Ready-to-Glow Secreted Luciferase System eliminates these obstacles.

Enhanced benefits of the Ready-To-Glow Secreted Luciferase System

The *Metridia longa* secreted luciferase gene, which was cloned from the marine copepod *Metridia longa,* encodes a 24 kDa protein containing an N-terminal secretory signal peptide of 17 amino acid residues (1). This secreted luciferase gene was sequence-optimized by deleting possible cis-acting sites (splice sites) and increasing the overall GC content to prolong mRNA half-life. The sequence was also human codon-optimized.

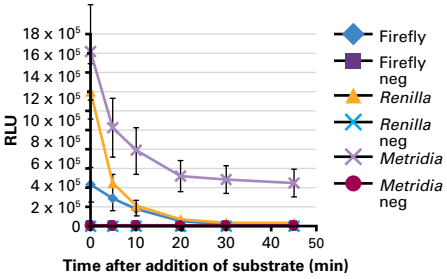

Figure 1. High signal intensity and stability using secreted *Metridia* luciferase. CHO cells were plated into 96-well plates and transiently transfected with CMV-driven constructs encoding non-secreted firefly luciferase, non-secreted *Renilla* luciferase, and sequence-optimized secreted *Metridia* luciferase. 24 hr after transfection, luciferase activity in equivalent amounts of samples were analyzed by addition of the recommended substrate. The signal was measured at different timepoints over a period of 45 min. neg = negative control.

Some of the key advantages of our Ready-To-Glow Secreted Luciferase System are highlighted by several critical experiments. One such experiment (Figure 1) shows that *Metridia* secreted luciferase exhibits a higher signal stability after addition of substrate compared to other non-secreted luciferase reporters such as *Renilla* luciferase and firefly luciferase, without compromising signal intensity, in the presence of 10% FCS in the media supernatant. This allows easy handling of multiple samples at the same time.

To test the performance of the secreted *Metridia* luciferase gene as a promoter response element reporter, the NFκB response element was cloned into the MCS (multiple cloning site) of the pMetLuc-Reporter Vector and transfected into HeLa cells. Six hours after induction of transiently transfected cells with TNF-α, activation of the NFκB response element was detected by assaying the cell supernatant for *Metridia* secreted luciferase activity. Although signal intensity after substrate addition decreased with time, the overall fold induction remained the same 30 minutes after substrate addition (Figure 2).

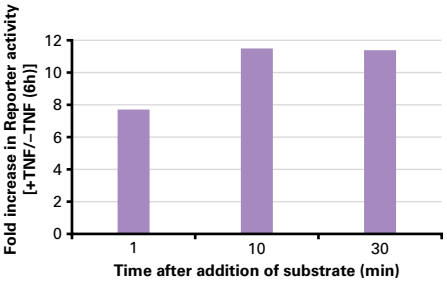

Figure 2. Monitoring promoter activation using the sequence-optimized secreted *Metridia* luciferase reporter. HeLa cells were transiently transfected with a vector construct containing the NFκB response element driving the expression of sequence-optimized secreted *Metridia* luciferase. 24 hr after transfection, the media was removed and replaced by media with or without TNF-α (100 ng/ml) to activate the NFκB signal transduction pathway. Six hr after addition of TNF-α, samples of the media were removed and analyzed for *Metridia* luciferase activity. The fold induction was calculated for different time points following the addition of substrate.

High-throughput applications

Ease of use, elimination of cell lysis, signal stability, and high signal intensity make Ready-To-Glow Secreted Luciferase System a powerful tool for high-throughput applications. As shown in Figure 3 (courtesy of Bayer Health Care, Germany), images of CHO cells in a 1,536-well format, stably expressing *Metridia* secreted luciferase under the control of a forskolin inducible promoter element, were visualized using a regular, low sensitivity CCD camera. These data confirm that the Ready-To-Glow Secreted Luciferase System is well-suited for high-throughput applications.

Kit components & features

Our **Ready-To-Glow Secreted Luciferase Reporter System** consists of two separate

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Ready-To-Glow™ Secreted Luciferase System...continued

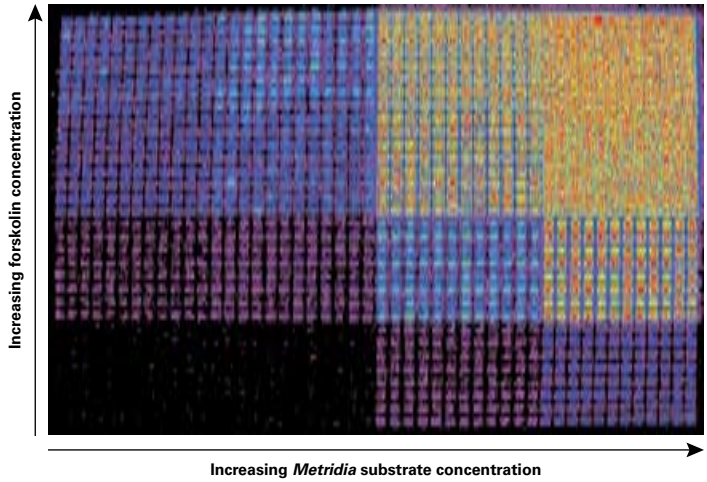

Figure 3. Use of secreted *Metridia* luciferase in a High-Throughput Screening (HTS) application. The figure shows a screen shot from a CCD camera of a 1,536-well plate containing stable CHO cells transfected with a forskolin-responsive *Metridia* luciferase gene (pASM-Lu164). 300 cells/well were plated in a 1,536-well microtiter plate. Cells were incubated with increasing concentrations of forskolin and the plate was incubated for 4 hr at 37° C. *Metridia* substrate was added in increasing concentrations and the plate was visualized using a CCD camera system (Integration time: 60 sec). (Courtesy of Bayer Health Care, Germany). This pseudocolor image reflects luminescence intensity. Yellow regions are the brightest, while black regions are the least bright.

Product	Size	Cat. No.
Ready-To-Glow Secreted Luciferase pMetLuc Vector Kit	each	631729
Ready-To-Glow Secreted Luciferase Reporter Assay	100 rxns	631726
	500 rxns	631727
	1, 000 rxns	631728
Ready-To-Glow Secreted Luciferase Reporter System[1]	100 rxns	631730
	500 rxns	631731
	1,000 rxns	631732

1 The Reporter System combines the Reporter Assay and the pMetLuc Vector Kit components.

Secreted Luciferase Reporter Assay Components
- Substrate Buffer
- Reaction Buffer
- Lyophilized Secreted Luciferase Substrate

Secreted Luciferase pMetLuc Vector Kit Components
- pMetLuc-Control Vector
- pMetLuc-Reporter Vector

Related Products
- Fusion-Blue™ Competent Cells (Cat. Nos. 636700 & 636758)
- Supercharge EZ10 Competent Cells (Cat. No. 636756)

1. **Clone response element/ promoter of interest into pMetLuc-Reporter vector**
2. **Transfect host cell line**
3. **At desired time points, assay luciferase activity**
   - Transfer media sample to 96-well plate
   - Add substrate reaction buffer
   - Assay luciferase activity in a luminometer

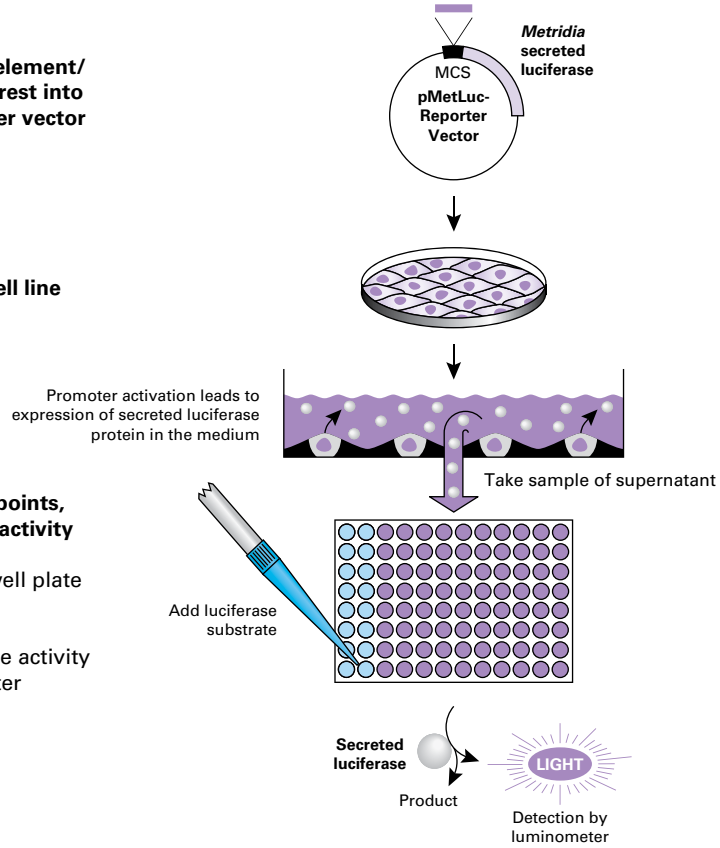

Figure 4. Flow chart of the Ready-To-Glow Secreted Luciferase Reporter System.

kits—the **Ready-To-Glow Secreted Luciferase Reporter Assay** and the **Ready-To-Glow Secreted Luciferase pMetLuc Vector Kit** (which also includes a control vector). The reporter assay and vector are also available separately. The pMetLuc-Reporter Vector is a promoterless vector designed to insert your promoter of interest upstream of the sequence-optimized *Metridia* secreted luciferase reporter gene. Detection is made simple with our Ready-To-Glow Secreted Luciferase Reporter Assay, which has been optimized for use together with our Ready-To-Glow Secreted Luciferase pMetLuc Vector Kit. A working scheme of the entire protocol is outlined in Figure 4.

Reference

1. Markova S. V., *et al. J. Biol. Chem.* **279**(5):3212–3217.