Ready-To-Glow™ Secreted Reporter Systems—FAQs

Simple—Powerful—Flexible

- **Homogeneous live cell assay**
- **Multiplex reporters**
- **Conduct multiple experiments using the exact same cells**

Clontech's **Ready-To-Glow Secreted Luciferase System** is ideal for studying the interactions between transcription factors and specific promoters/response elements, an important stage in signal transduction. The highly sensitive enzymatic reporter is measured in the supernatant of transfected cells—without cell lysis (Figure 1).

Frequently Asked Questions

1. Why use a secreted reporter assay?

You eliminate the lysis step required for most reporter assays, which significantly decreases your time and effort. Even more importantly, no lysis means live cells—and no experimental dead ends because you had to sacrifice your sample. Complete a time course study without sample-to-sample variability, or study different inducers or repressors. Then pursue additional experiments, using the same sample.

2. Why does a secreted reporter assay reduce the background signal?

In some cases, your promoter of interest may be "leaky", and so the reporter is expressed and accumulated between the time of transfection and the actual experiment. If you are using a nonsecreted reporter, it accumulates inside the cell, and therefore cannot be removed. However, if the reporter is secreted, a simple media change before starting your experiment will remove any reporter that may have accumulated since the initial transfection.

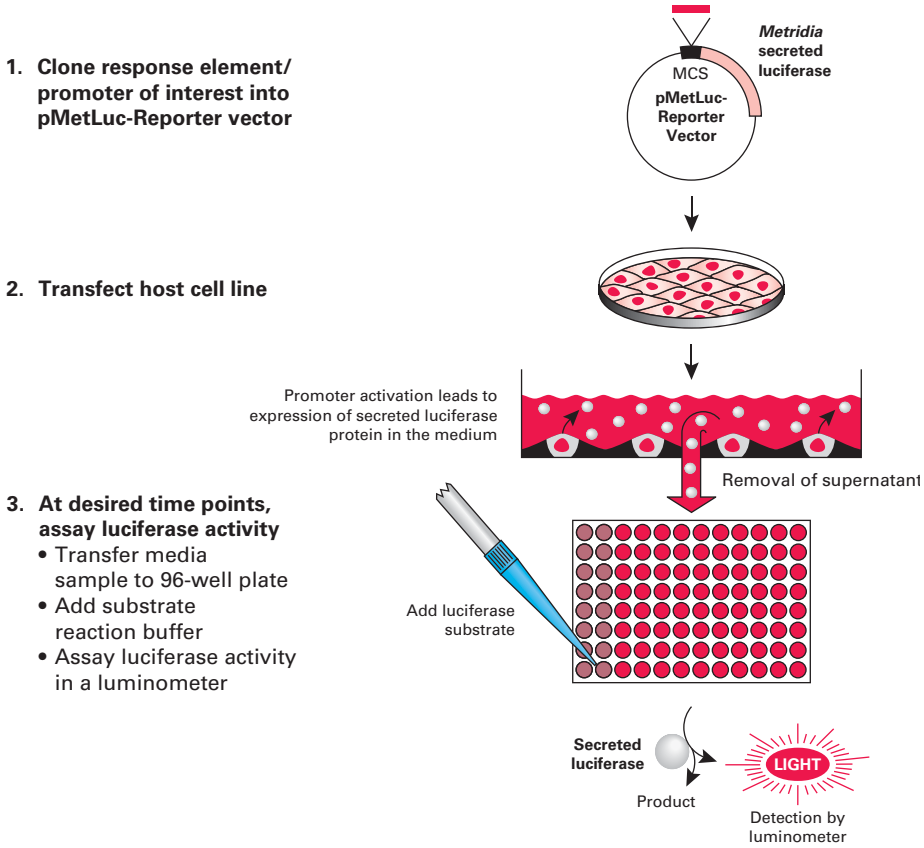

Figure 1. Flow chart of the Ready-To-Glow Secreted Luciferase Assay.

3. What is the limit of detection for secreted *Metridia* luciferase? What is the minimum number of cells required to detect *Metridia* luciferase?

Metridia luciferase activity is linear over a very broad range of concentration—at least 6 logs. The limit of detection has been determined to be 2 fg/well, or 40 fg/ml (1; Figure 2). As few as 200–300 cells expressing *Metridia* luciferase can be detected in one well of a 96-well plate.

4. Is *Metridia* luciferase stable in the presence of DMSO?

Yes, *Metridia* luciferase is stable in the presence of up to 2% DMSO (data not shown).

Metridia luciferase is also very stable at 37°C. Even after incubation at 37°C for 72 hours, *Metridia* luciferase maintains 85% of its original activity (data not shown).

Ready-To-Glow™ FAQs...continued

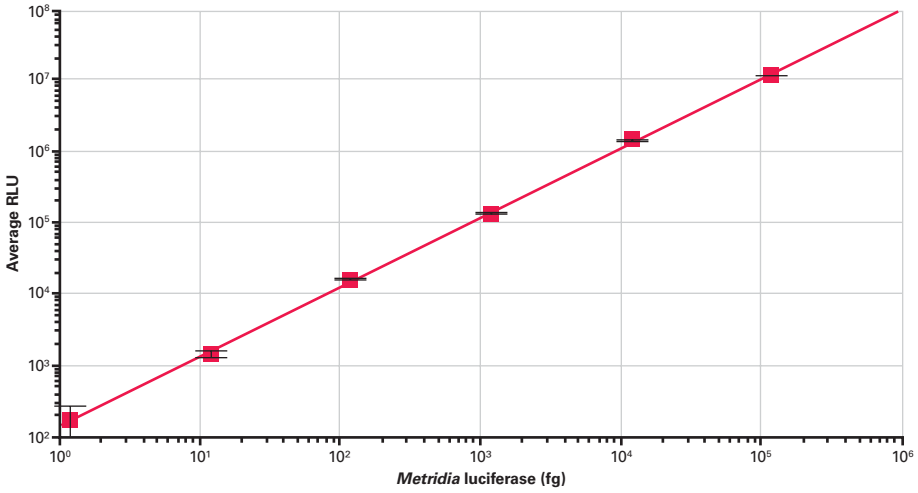

Figure 2. Limit of detection for recombinant *Metridia* luciferase protein. The indicated amounts of recombinant *Metridia* luciferase (fg/well) were spiked into DMEM containing 10% FBS. *Metridia* luciferase activity was measured in a 96-well format on the SpectraMax® L. The limit of detection was determined to be 2 fg/well (40 fg/ml).

Product	Size	Cat. No.
Ready-To-Glow Secreted Luciferase pMetLuc Vector Kit		
	20 µg	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
Ready-To-Glow Dual Secreted Reporter Vector Kit		
	4 x 20 µg	631735
Ready-To-Glow Dual Secreted Reporter Assay		
	500 rxns	631734

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

5. What kind of plates can I use to assay for *Metridia* luciferase?

96-, 384-, and 1,536-well plates have all been used successfully for Ready-To-Glow assays (1, 2). As expected with all chemiluminescent assays, clear plates are not optimal due to cross talk between neighboring wells. We strongly suggest using white-walled plates; we typically obtain 3.5-fold higher readings (RLU) in white-walled plates compared to clear plates. Black-walled plates can also be used; however the total RLUs are lower than for white-walled plates (data not shown).

6. How do I perform the Ready-To-Glow assay?

The Ready-To-Glow assay is simple and straightforward (Figure 1). Since it is also homogeneous, you can perform the assay directly in the plate with your cells and culture medium, or you can transfer a media sample into a separate plate. Performing the assay in the plate with the cells may streamline your experiment, and makes it easy to adapt the assay for high-throughput applications (2).

7. How do I multiplex Ready-To-Glow with other reporters?

Clontech offers a **Ready-To-Glow Dual Secreted Reporter System,** which includes a secreted form of human placental alkaline phosphatase (SEAP) in addition to *Metridia* luciferase (3). You can cotransfect your cells with both reporters, and stimulate them to express and secrete *Metridia* luciferase or SEAP, or both. Since both reporters are secreted, you can monitor promoter-specific activity from each reporter directly from the culture medium. You can test two promoters/response elements, or use one reporter to measure transfection efficiency.

You can also multiplex Ready-To-Glow with fluorescent reporters: multimode plate readers can accommodate fluorescence, chemiluminescence, and absorption readings, allowing you to "mix and match" reporters according to your experimental needs. Simply transfect with the appropriate vectors.

8. What can I do downstream?

Since your cells are alive, you can get additional data from the same culture. Perform a kinetic study, test several potential inducers/repressors, test toxicity/viability, perform microscopy, immunocytochemistry, protein or RNA analysis…whatever continues and enhances your research.

References

1. Detection Limit and Linear Range of Ready-To-Glow™ Secreted *Metridia* Luciferase Determined on the Molecular Devices SpectraMax L. (July 2007) *Clontechniques* **XXII**(3):20–21.
2. Ready-To-Glow™ Secreted Luciferase System. (July 2006) *Clontechniques* **XXI**(2):12–13.
3. Ready-To-Glow™ Dual Secreted Reporter System. (October 2006) *Clontechniques* **XXI**(3):1.