Protocol-at-a-Glance

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Ready-To-Glow™ Secreted Luciferase Reporter Assay Protocol-at-a-Glance

(PT3902-2)

This protocol is provided for use with the Ready-To-Glow Secreted Luciferase Reporter Assay (Cat. Nos. 631726, 631727 & 631728). For a general introduction to the theory of the Ready-To-Glow Secreted Luciferase Reporter System, please refer to User Manual PT3902-1, which can be found at **www.clontech.com/support/manuals.asp**. For Information on the *In Vivo* Luciferase Imaging Kit, visit **www.clontech.com/ imaging.**

For transient transfection assays, secreted *Metridia* luciferase is generally detected in the medium 12–18 hours after transfection, with maximal levels detected between 48–72 hours. Optimal times will vary depending on the cell type, cell density, and the particular experimental conditions. Each construct should be transfected and assayed in triplicate.

1. **Prepare Reagents and Samples for the Secreted *Metridia* Luciferase Assay**
   a. *Prepare 10X Substrate Stock Solution*: Dissolve the Lyophilized Secreted Luciferase Substrate in the total volume of Substrate Buffer supplied with the kit. Gently mix the substrate in the Substrate Buffer by pipetting. Avoid generating air bubbles; **do not agitate or vortex**.

   b. *Prepare 1X Substrate/Reaction Buffer:* Dilute 10X Substrate Stock Solution 1:10 in Reaction Buffer to make the required amount of 1X Substrate/Reaction Buffer. To calculate the total volume of 1X Substrate/Reaction Buffer required, multiply the number of samples by a factor of 5. (For example, for 20 samples, you would prepare 100 µl of 1X Substrate/Reaction Buffer by diluting 10 µl of 10X Substrate Stock Solution in 90 µl of Reaction Buffer.)

   c. Mix the 1X Substrate/Reaction Buffer gently, with slow pipetting. **Do not vortex.** Allow the 1X Substrate/Reaction Buffer to remain at room temperature for 10 min prior to use.

   d. Transfer 50 µl of cell culture medium from transfected cells or mock transfected cells (in triplicate) to a 96-well microtiter plate. If necessary, the plate can be frozen at –20°C for future analysis.

   We recommend Microlite™ 1 Luminescence Microtiter 96-well plates (VWR Cat. No. 62403-124).

2. **Perform the Secreted *Metridia* Luciferase Assay**
   a. Add 5 µl of 1X Substrate/Reaction buffer to each sample. If a large number of samples are assayed, use a multichannel pipette to reduce the time between substrate addition and signal detection.

   b. Transfer the plate to a luminometer and record light signals according to the manufacturer's recommended luminometer settings. Refer to your plate reader's user manual for additional information regarding its performance and use.

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Notice to Purchaser

Metridia Luciferase: Markova, S. V., Golz, S., Frank, L. A., Kalthof, B. & Vysotski, E. S. (2004) Cloning and expression of cDNA for a luciferase from the marine copepod *Metridia longa*. A novel secreted bioluminescent reporter enzyme. *J. Biol. Chem.* **279**(5):3212–3117.

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