Protocol-at-a-Glance

Ready-To-Glow™ Automation Kit
Protocol-at-a-Glance
(PT4049-2)

This protocol is provided for use with the Ready-To-Glow Automation Kit (Cat. Nos. 631739 & 631740). For detailed background information about the Ready-To-Glow Secreted Luciferase System, please refer to its User Manual, PT3902-1, which can be found at **www.clontech.com/support/**. For the specifics of the Automation Kit protocol, refer only to this Protocol-at-a-Glance.

When to Perform the Ready-To-Glow Assay

For transient transfection assays using the pMetLuc control vector, 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 Samples for the Ready-To-Glow Assay

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

Note: If you prefer to grow the cells and carry out the reading in the same plate, we recommend using clear bottom plates with white walls, such as Nunc™ Microwell 96-Well Optical Bottom Plates (Nunc Cat. No. 165306).

2. Prepare 100X Substrate Stock Solution

Dissolve the Lyophilized Secreted Luciferase Substrate in 500 µl (1,000 rxn kit) or 2.5 ml (5,000 rxn kit) of Substrate Buffer. Gently mix to dissolve the substrate in the Substrate Buffer by pipetting gently.

Important: The substrate is sensitive to oxidation in the presence of air bubbles caused by agitation. DO NOT AGITATE OR VORTEX to dissolve the substrate.

3. Prepare 1X Substrate/Reaction Buffer

a. Multiply the number of samples by a factor of 50. This is the volume of 1X Substrate/Reaction Buffer required.

b. Dilute 100X Substrate Stock Solution (prepared in Step 2) 1:100 in Reaction Buffer to prepare the required amount of 1X Substrate/Reaction Buffer.

For example, for 100 samples, you would prepare 100 x 50 = 5,000 µl of 1X Substrate/Reaction Buffer by diluting 50 µl of 100X Substrate Stock Solution in 4,950 µl of Reaction Buffer.

c. Mix the 1X Substrate/Reaction Buffer gently, with slow pipetting. DO NOT VORTEX to mix. Allow the 1X Substrate/Reaction Buffer to remain at room temperature for 10 minutes prior to use.

4. Perform the Ready-To-Glow Assay

a. Add 50 µl of 1X Substrate/Reaction buffer to each sample, either by pipetting it into the wells manually or via an automatic injector system connected to your plate reader.

b. Detect and record the Secreted *Metridia* Luciferase signal according to your plate reader's user manual.

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Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

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Products containing *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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