Protocol-at-a-Glance

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

(PT3940-2)

This protocol is provided for use with the Ready-To-Glow Dual Secreted Reporter Assay (Cat. No. 631734). For a general introduction to the theory of each reporter system, please refer to the User Manuals, which can be found at **www.clontech.com/support/manuals.asp** (PT3057-1 for the Great EscAPe™ SEAP Reporter System and PT3902-1 for the Ready-To-Glow Secreted Luciferase Reporter System). For the specifics of the Dual Secreted Reporter protocol, refer only to this Protocol-at-a Glance.

A. Chemiluminescent SEAP Reporter Assay

For transient transfection assays using the pSEAP2-Control vector, a secreted form of human alkaline phosphatase (SEAP) 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 SEAP Assay

a. Transfer 25 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 Scientific Products, Cat. No. 62403-124).

b. Prepare 1X Dilution Buffer by diluting the 5X Dilution Buffer 1:5 with ddH_2O.

2. Perform the SEAP Assay

You may need to dilute some samples in order to stay within the linear range of the assay. To determine the linear range, assay a dilution series of your sample and a dilution series of recombinant human placental secreted alkaline phosphatase before you assay your samples.

a. Allow the SEAP Substrate Solution to equilibrate to room temperature (22–25°C).

b. Add 75 µl of 1X Dilution Buffer to each sample (from Step 1.a.) in the 96-well microtiter plate.

c. Seal the plate with adhesive aluminum foil or a 96-well plate lid and incubate the diluted samples for 30 min at 65°C using a heat block or water bath.

d. Cool the samples on ice for 2–3 min, then equilibrate to room temperature.

e. Add 100 µl of SEAP Substrate Solution to each sample. Incubate for 10–60 min (30 min recommended) at room temperature before reading.

f. Use a 96-well plate reader luminometer to detect and record the SEAP signal. Optimal readings will be obtained 10–60 min after substrate addition.

   Refer to your plate reader's user manual for additional information regarding its performance and use.

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B. Secreted *Metridia* Luciferase Reporter Assay

For transient transfection assays using the pMetLuc2-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 Reagents and Samples for the Secreted *Metridia* Luciferase Assay

a. 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).

b. *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. (The substrate is sensitive to oxidation in the presence of air bubbles caused by agitation. DO NOT AGITATE OR VORTEX to dissolve the substrate.)

c. *Prepare 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. Dilute 10X Substrate Stock Solution 1:10 in Reaction Buffer to make the required amount of 1X Substrate/Reaction Buffer. (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.)

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 min prior to use.

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. Use a 96-well plate reader luminometer to detect and record the Secreted *Metridia* Luciferase signal.

Refer to your plate reader's user manual for additional information regarding its performance and use.

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.