Protocol-at-a-Glance

# Great EscAPe™ SEAP Chemiluminescence Kit 2.0
# Protocol-at-a-Glance
**(PT3954-2)**

This protocol is provided for use with the Great EscAPe SEAP Chemiluminescence Kit 2.0 (Cat. Nos. 631736, 631737, and 631738). For a general introduction to the theory of the SEAP reporter system, please refer to the Great EscAPe™ SEAP User Manual (PT3057-1), which can be found at **www.clontech.com**. For the specifics of the Great EscAPe SEAP Chemiluminescence Kit 2.0 protocol, refer only to this Protocol-at-a Glance.

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 hr after transfection, with maximal levels detected between 48–72 hr. 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.

## A. Prepare Reagents and Samples for the SEAP Assay

1. 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 sealed and frozen at –20°C for future analysis.

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

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

## B. 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.

1. Allow the SEAP Substrate Solution to equilibrate to room temperature (22–25°C).
2. Add 75 µl of 1X Dilution Buffer to each sample (from Step A.1) in the 96-well microtiter plate.
3. Seal the plate with adhesive aluminum foil or a regular 96-well lid and incubate the diluted samples for 30 min at 65°C using a heat block or water bath. Seal well and use extra care when incubating in a water bath.
4. Cool the samples on ice for 2–3 min, then equilibrate to room temperature.
5. Add 100 µl of SEAP Substrate Solution to each sample. Incubate for 10–60 min (30 min is recommended) at room temperature before reading.
6. Use a 96-well plate reader luminometer to detect and record the SEAP signal. Optimal readings will be obtained from 10–60 min after substrate addition.

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

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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