Protocol-at-a-Glance

Great EscAPe™ SEAP Reporter System 3
Protocol-at-a-Glance
(PT3057-2)

This abbreviated protocol is provided for your convenience. Please read through the User Manual (PT3057-1) before using the Protocol-at-a-Glance.

Each construct should be transfected and subsequently assayed in triplicate. It may be necessary to dilute some samples in Sections B or C in order to stay within the linear range of the assay. The linear range can be determined by assaying a dilution series of the positive control placental alkaline phosphatase.

A. Preparation of Samples for Assay of SEAP

For transient transfection assays, maximal levels of SEAP are generally detected in the medium 48–72 hours after transfection; optimal times will vary depending on the cell type, cell density, and the particular experimental conditions.

1. Remove 110 µl of cell culture medium and transfer to a microcentrifuge tube.
2. Centrifuge at 12,000 x g for 10 sec to pellet any detached cells present in the culture medium.
3. Transfer 100 µl of supernatant to a fresh microcentrifuge tube.
4. Store at –20°C until ready for assay.

B. Chemiluminescent SEAP Assay

1. Allow the necessary amount of Chemiluminescent Enhancer and Assay Buffer to equilibrate to room temperature (22–25°C).
2. Prepare 1X Dilution Buffer, and allow to equilibrate to room temperature.
3. Thaw samples of culture medium, and place 25 µl into a 0.5-ml transparent microcentrifuge tube.
4. Add 75 µl of 1X Dilution Buffer to each 25-µl sample and mix gently.
5. Incubate the diluted samples for 30 min at 65°C using a heat block or water bath.
6. Cool samples by placing on ice for 2–3 min, then equilibrate to room temperature.
7. Add 100 µl of Assay Buffer to each sample and incubate for 5 min at room temperature.
8. Prepare 1.25 mM CSPD Substrate by diluting 1:20 with Chemiluminescent Enhancer (Section II in User Manual).
9. Add 100 µl of the diluted substrate to each tube, and incubate for at least 10 min at room temperature. Optimal readings will be obtained 10–60 min after substrate addition.

Chemiluminescence Detection Methods

If the assay is not performed in a suitable tube, transfer the entire solution from Step B.9 to the appropriate luminometer tube and place in the instrument. Record light signals as 5- to 15-second integrals.

Consult the main protocol for information on detection using a plate luminometer or x-ray film.

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C. Fluorescent SEAP Assay

Note: This format is for 96-well plates. If performing the assay in microcentrifuge tubes, the volumes can be increased.

1. Allow a sufficient amount of assay buffer for the entire experiment to equilibrate to room temperature (22–25°C).
2. Prepare the required amount of 1X Dilution Buffer, and allow to equilibrate to room temperature.
3. Thaw samples of cell culture medium, and place 25 µl of each sample into a separate well of a 96-well plate.
4. Add 25 µl of 1X Dilution Buffer to each 25 µl sample and mix gently.
5. Incubate the diluted samples for 30 min in a 65°C incubator. Be sure to put the lid on 96-well plates. (If you are performing the assay in microcentrifuge tubes, you can use a heat block or water bath.)
6. Cool samples to room temperature by placing on ice for 2–3 min, and then equilibrating to room temperature.
7. Add 97 µl of Assay Buffer to each sample and incubate for 5 min at room temperature.
8. Prepare enough of a 1 mM MUP working dilution for your experiment by diluting the 10 mM MUP Fluorescent Substrate (1:10) in 1X Dilution Buffer.
9. Add 3 µl of the 1 mM MUP working dilution to each sample, and incubate for 60 min ***in the dark*** at room temperature.

Fluorescence Detection Methods

The excitation and emission peaks of MUP fluorescence are 360 nm and 449 nm, respectively.

- Detection using a plate fluorometer

  If the assays have been performed in a 96-well flat bottom microtiter plate suitable for plate fluorometers (i.e., black walls, transparent bottom), fluorescence can be measured directly using a PerSeptive Biosystems Cytofluor II Fluorescence Multiwell Plate Reader with the gain set at 58–68.

- Detection using a tube fluorometer

  If the assay is performed in a tube suitable for fluorometer readings the sample may be placed directly in the instrument after Step 9. If the assay is not performed in a suitable tube, transfer the entire solution from Step 9 to the appropriate tube and place in the instrument.