Great EscAPe™ SEAP User Manual

PT3057-1 (PR55697)
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I. Introduction

The **Great EscAPe™ SEAP Reporter System 3** and the **Great EscAPe™ SEAP Chemiluminescence and Fluorescence Detection Kits** are versatile tools for the systematic analysis of eukaryotic promoters and enhancers. The Great EscAPe system uses SEAP—a secreted form of human placental alkaline phosphatase (Berger et al., 1988)—as a reporter molecule to monitor the activity of promoters and enhancers. Such sequences can be cloned into the pSEAP2-Basic Vector, which is included in the Reporter System 3 and is also available separately. The chemiluminescent substrate CSPD (PubChem CID No. 424756) and the fluorescent substrate, 4-methylumbelliferyl phosphate (MUP), enable researchers to monitor expression of the SEAP reporter gene using simple, sensitive, nonradioactive assays of secreted phosphatase activity (see Figure 1). The chemiluminescent assay can detect as little as 10^{-13} g of SEAP protein, making it one of the most sensitive enzymatic reporters available. In side-by-side assays, the fluorescent assay is 10- to 100-fold less sensitive than the chemiluminescent assay, but comparable to assays for firefly luciferase and suitable for all but the most demanding systems. Both assays are linear over a 10^4–fold range of enzyme concentrations, making them particularly well suited for comparative analyses.

The SEAP reporter gene encodes a truncated form of the placental enzyme that lacks the membrane anchoring domain, thereby allowing the protein to be efficiently secreted from transfected cells. Changes in levels of SEAP activity detected in the culture medium have been shown to be directly proportional to changes in intracellular concentrations of SEAP mRNA and protein (Berger et al., 1988; Cullen & Malim, 1992). SEAP has the unusual properties of being extremely heat stable and resistant to the phosphatase inhibitor L-homoarginine (Cullen & Malim, 1992). Therefore, endogenous alkaline phosphatase activity can be eliminated by pretreatment of samples at 65°C and incubation with this inhibitor.

The secreted nature of SEAP provides several advantages for the use of this enzyme as a transcription reporter:

- Preparation of cell lysates is not required for analysis.
- The kinetics of gene expression can be studied simply by repeated collection of the culture medium from the same cultures.
- Transfected cells are not disturbed by measurement of SEAP activity in the medium, so a single set of cultures can be used both for the SEAP assay and for further investigations such as RNA and protein analysis.
- Background from endogenous alkaline phosphatase is almost absent in the culture medium following pretreatment.
- Sample collection from the culture medium can be automated by growing cultures and performing the assays in 96-well plates.

I. Introduction continued

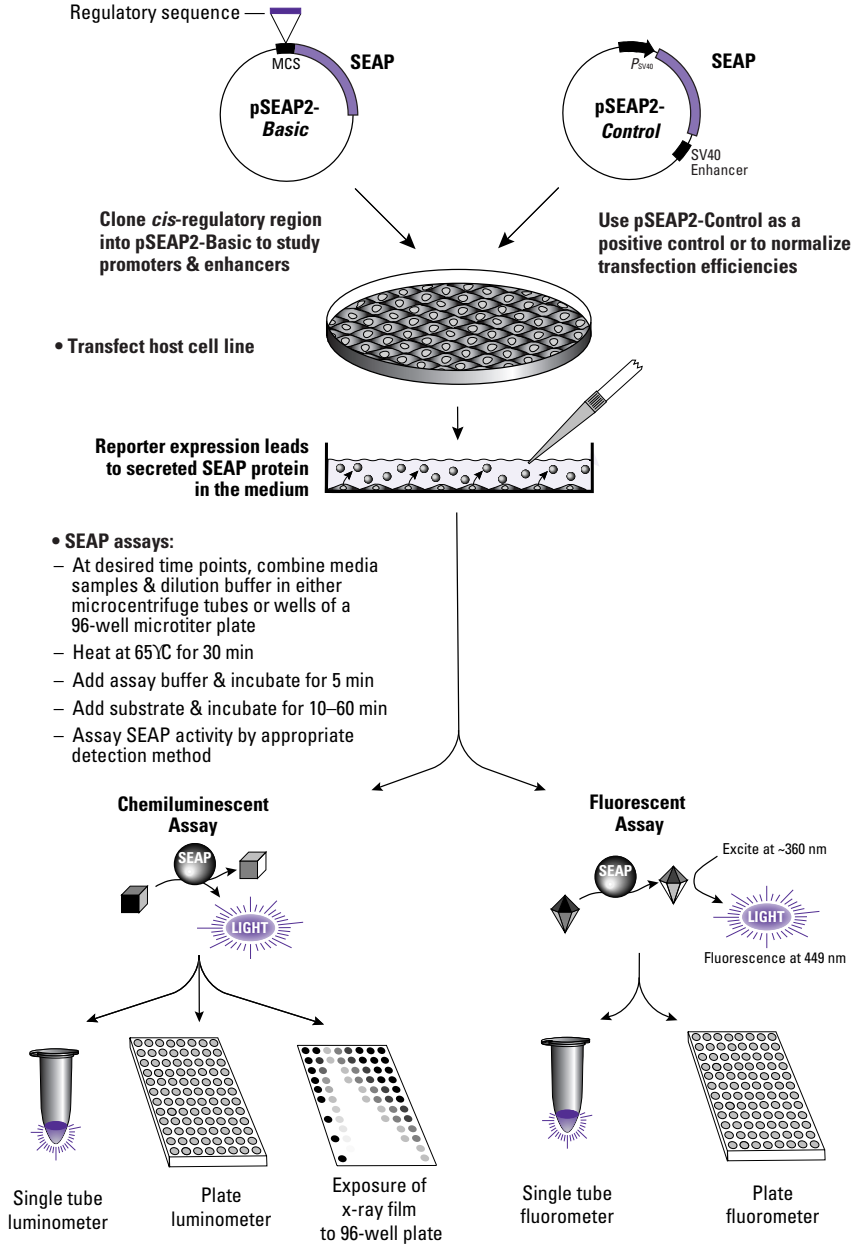

Figure 1. Flowchart of the Great EscAPe™ SEAP Assay procedure.

I. Introduction continued

Great EscAPe™ SEAP Reporter Vectors

The Great EscAPe SEAP Reporter System 3 includes two SEAP Reporter Vectors, which have been designed to provide maximal flexibility in studying regulatory sequences from the gene of interest. For vector maps and multiple cloning site (MCS) sequences, see Appendix A or visit our web site at **www.clontech.com**.

- **pSEAP2-Basic** lacks eukaryotic promoter and enhancer sequences, and it contains an MCS that allows promoter DNA fragments to be inserted upstream of the SEAP gene. Enhancers can be cloned into either the MCS or unique downstream sites.
- **pSEAP2-Control** is pSEAP2-Basic with the SV40 early promoter inserted upstream of the SEAP gene and the SV40 enhancer inserted downstream. pSEAP2-Control expresses SEAP in most cell types and provides an important positive control; in particular, it can be useful for normalizing transfection efficiencies.

The pSEAP2 Vectors incorporate a number of improvements over the original Great EscAPe vectors, such as including an enhanced Kozak consensus translation initiation site (Kozak, 1987); and removing the SV40 small-t intron, which would permit cryptic splicing and reduced expression in some genes and/or cell types (Huang & Gorman, 1990; Evans & Scarpulla, 1989). Additional improvements include switching from the early to late polyadenylation signal of SV40, which typically causes a fivefold increase in mRNA levels (Carswell & Alwine, 1989); and removing extraneous sequences from the 3' untranslated region of the SEAP mRNA. A synthetic transcription blocker is located upstream of the SEAP transcription unit and reduces background transcription (Eggermont & Proudfoot, 1993). The new vectors are also smaller and feature expanded multiple cloning sites.

II. List of Components

The pSEAP2 Vectors and the Positive Control Placental Alkaline Phosphatase should be stored at –20°C. All other components of the Great EscAPe kits should be stored at 4°C.

The **Great EscAPe™ SEAP Reporter System 3** (Cat. No. 631706) contains sufficient reagents for 200 SEAP assays (100 chemiluminescent assays and 100 fluorescent assays). The **Great EscAPe™ SEAP Chemiluminescence Detection Kits** (Cat. Nos. 631702 & 631701) and the **Great EscAPe™ SEAP Chemiluminescence 1,000 Rxns High-Throughput** detection kit (Cat. No. 631725) contain sufficient reagents for 50, 300, and 1,000 SEAP assays, respectively. The **Great EscAPe™ SEAP Fluorescence Detection Kit** contains sufficient reagents for 300 SEAP assays (Cat. No. 631704).

Cat No. 631706	Cat No. 631702	Cat No. 631701	Cat No. 631725	Cat No. 631704	
10 µg	- -	- -	- -	- -	**pSEAP2-Basic Vector**
10 µg	- -	- -	- -	- -	**pSEAP2-Control Vector**
0.6 ml	0.4ml	1.8 ml	3.6 ml	- -	**CSPD Chemiluminescent Substrate** (25 mM) Store in the dark at 4°C. A final concentration of 1.25 mM is recommended for chemiluminescent detection of SEAP activity. Prepare substrate solution as follows just prior to use: 25 mM CSPD 1 vol.* Chemiluminescent Enhancer 19 vol.*
12 ml	6 ml	36 ml	72 ml	- -	**Chemiluminescent Enhancer**
0.1 ml	- -	- -	- -	0.3 ml	**MUP Fluorescent Substrate** (10 mM) Store in the dark. Dilute 1:10 in 1X Dilution Buffer prior to use
24 ml	6 ml	36 ml	72 ml	36 ml	**Assay Buffer** Contains the alkaline phosphatase inhibitor L-homoarginine.
10 ml	4 ml	20 ml	40 ml	20 ml	**5X Dilution Buffer** Prepare 1X Dilution Buffer as follows just prior to use, and equilibrate to room temperature: 5X Dilution Buffer 1 vol.* ddH_2O 4 vol.*
30 µl	20 µl	50 µl	100 µl	50 µl	**Positive Control Placental Alkaline Phosphatase** Provided at a concentration of 0.1 mg/ml in 50% (v/v) glycerol, 2mM Na_2HPO_4 (pH 7.2)

* vol. = volume(s)

III. Additional Materials Required

Great EscAPe SEAP assays can be carried out either in 0.5 ml microcentrifuge tubes or 96-well microtiter plates (for high-throughput applications; see Section V).

Chemiluminescence detection of SEAP activity can be performed using either a tube or plate luminometer, or via exposure of x-ray film to reactions carried out in 96-well microtiter plates. Fluorescence detection of SEAP activity can be performed with either a tube or plate fluorometer.

The following types of microtiter plates and accessories are recommended for high-throughput applications:

- **Falcon™ 96-well PCR Reaction Plates, low profile** (Cat. No. 352134) These clear plates, which contain conical wells, are recommended for sample preparation.
- **Microlite 1 Luminescence Microtiter 96-well plates, Thermo Electron, Flat-Bottom** (VWR Scientific Products, Cat. No. 62403-124).
  These opaque white plates, which contain flat-bottom wells, are recommended for chemiluminescent assays and detection.
- **AluminaSeal™ PreCut Adhesive Foil Sealing Film** (Diversified Biotech, Cat. No. ALUM-1000). This film is recommended for sealing both types of 96-well plates.

IV. Experimental Design and Sample Preparation

PLEASE READ THROUGH ENTIRE PROTOCOL BEFORE BEGINNING

A. Transfection of Mammalian Cells with SEAP Expression Vectors

1. Transfection techniques

   The pSEAP2 Vectors may be transfected into mammalian cells by a variety of techniques, including those using calcium phosphate (Chen & Okayama, 1988), DEAE-dextran, various liposome-based transfection reagents (Kain, 1996), and electroporation. For high efficiency transfections with relatively short transfection times, we recommend using the CalPhos™ Mammalian Transfection Kit (Cat. No. 631312) or Clonfectin™ Transfection Reagent (Cat. No. 631301), for calcium phosphate and liposome-based transfections, respectively. These methods are compatible with both SEAP assays, so the procedure of choice will depend primarily on the type of cell being transfected. Different cell lines may vary by several orders of magnitude in their ability to take up and express exogenous DNA. Moreover, a method that works well for one type of cultured cell may be inferior for another. When working with a cell line for the first time, compare the efficiencies of several transfection protocols using the pSEAP2-Control Vector as described in Section B.

2. Transfection considerations

   a. Perform transfections in triplicate

      Each different construct should be transfected (and subsequently assayed) in triplicate to minimize variability among treatment groups. The primary sources of such variability are differences in transfection efficiencies.

   b. Normalize for transfection efficiency

      When monitoring the effect of promoter and enhancer sequences on gene expression, it is critical to include an internal control that will distinguish differences in the level of transcription from variability in the efficiency of transfection (Sambrook & Russell, 2001). This is easily done by cotransfecting a second plasmid that constitutively expresses an activity that can be clearly differentiated from SEAP. The level of the second enzymatic activity can then be used to normalize the levels of SEAP among different treatment groups. Reporter proteins frequently used for this purpose include E. coli β-galactosidase, pCMV-βgal, human growth hormone (hGH), fluorescent proteins, (pAcGFP1-N1, Cat. No. 632469), and luciferase.

   Note on effects of SV40 large T antigen (COS cells): The specific level of expression for the pSEAP2 Vectors is likely to vary in different cell types. This is particularly true for cell lines containing the SV40 large T antigen, such as COS cells. The large T antigen promotes replication

IV. Experimental Design and Sample Preparation continued

of the SV40 origin, sequences that are found in the promoter region of the pSEAP2-Control Vector. The combination of the large T antigen and SV40 origin leads to a higher copy number of these vectors in COS cells, which in turn may result in increased expression of the SEAP reporter gene relative to vectors lacking the SV40 origin.

B. Proper Use of Controls

1. Negative controls

   A negative control is necessary to measure the background signal associated with the cell culture media. This can be determined by assaying 25 µl of culture medium from cells transfected with the pSEAP2-Basic Vector, which contains the SEAP gene without a promoter or enhancer. The values obtained from such controls should be subtracted from experimental results.

2. Positive controls

   a. Positive control for transfection and expression of exogenous DNA

      A positive control is necessary to confirm transfection and expression of exogenous DNA and to verify the presence of active SEAP in the culture media. Expression and secretion of functional SEAP in transfected cells can be confirmed by assaying 25 µl of culture medium from cells transfected with the pSEAP2-Control Vector, which contains the SEAP structural gene under transcriptional control of the SV40 promoter and enhancer. Cells transfected with this plasmid should yield high activity within 48–72 hours after transfection.

   b. Positive control for detection method

      The provided Positive Control Placental Alkaline Phosphatase can be used to confirm that the detection method is working. To do this, simply add 2 µl of the Positive Control Alkaline Phosphatase to 23 µl of culture medium from untransfected cells. This should yield a strong positive signal. A dilution series of the positive control enzyme can also be used to determine the linear range of the assay.

3. Normalizing transfection efficiencies

   It is critical to include an internal control that will distinguish differences in the level of transcription from variability in the efficiency of transfection. See Section A.2.b or Sambrook & Russell (2001) for more information.

IV. Experimental Design and Sample Preparation continued

C. Staying Within the Linear Range of the Assay

It is important to stay within the linear response range of the assay. The linear range can be determined by assaying a dilution series of the Positive Control Placental Alkaline Phosphatase. High intensity signals can exceed the optimal range of detection instruments, resulting in incorrect, low readings. In addition, low intensity signals that are near background levels may fall outside the linear response range of the assay. Therefore, the target amount of SEAP in the assay should be adjusted to bring the signal within the linear range. For signals that are too intense, this can be achieved by diluting the cell culture media prior to assay. For low signals, the amount of SEAP may be increased by improving the transfection efficiency, starting with a greater number of cells, or increasing the volume of media assayed.

D. Sample Preparation for Chemiluminescent and Fluorescent SEAP Assays

For transient transfection assays, maximal levels of SEAP are generally detected in the cell culture medium between 48–72 hours after transfection. This range is suggested only as a starting point, as optimal times for collecting samples will vary for different cell types, cell densities, and the nature of the particular experimental conditions.

The following procedure should be used to prepare samples of conditioned medium for use in both chemiluminescent and fluorescent assays. This procedure is designed for use with adherent cells. If working with suspended cell cultures, simply begin with 125 µl of the cell culture, pellet the cells by centrifugation, and proceed from Step 1.

1. Remove 110 µl of conditioned cell culture medium (50 µl if cells were cultured in 96-well plates) and transfer to a microcentrifuge tube (or a fresh 96-well plate). Use 96-well plates with conical wells (see Section III) for sample preparation.
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 (40 µl if cells were cultured in 96-well plates) to a fresh microcentrifuge tube (or a fresh 96-well plate).
4. Cap tubes or cover plates with adhesive aluminum foil and store at –20°C until ready for assay.

V. Chemiluminescent SEAP Assay

A. Chemiluminescent SEAP Assay

As noted in Section IV, each construct should be transfected and subsequently assayed in triplicate. It may be necessary to dilute some samples in order to stay within the linear range of the assay. The linear range can be determined by assaying a dilution series of Positive Control Placental Alkaline Phosphatase.

When using 96-well microtiter plates (for detection with either a plate luminometer or x-ray film), use the smaller required volumes indicated in Steps 3, 4, 7, and 9 and also refer to the Great EscAPe™ SEAP Chemiluminescence High-Throughput Detection Kit Protocol- at-a-Glance (PT3835-2).

1. Allow enough Assay Buffer (see Step 7) and Chemiluminescent Enhancer (see Steps 8 and 9) for performing the entire experiment to equilibrate to room temperature.
2. Prepare the required amount of 1X Dilution Buffer (see Step 4) by diluting a sufficient volume of the provided 5X stock solution 1:5 with ddH_20 just prior to use (see Section II) and allowing it to equilibrate to room temperature.
3. Thaw samples of cell culture medium (from Step IV.D.4), and place 25 µl of each sample into a separate 0.5 ml transparent microcentrifuge tube. For 96-well plates, use 15 µl of culture medium per sample well. We recommend opaque white plates with flat-bottom wells (see Section III) for chemiluminescent assays and detection.
4. Add 75 µl of 1X Dilution Buffer to each 25 µl sample (in microcentrifuge tubes) and mix gently. For 96-well plates, add 45 µl of 1X Dilution Buffer to each sample well and mix gently using a vortex mixer with a plate adaptor.
5. Incubate the diluted samples (in microcentrifuge tubes) for 30 min at 65°C using a heating block or water bath. If using 96-well plate(s), be sure to seal the sample wells with adhesive aluminum foil (see Section III) and incubate the plates in a 65°C incubator for 30 min.
6. Cool samples to room temperature by placing on ice for 2–3 min, then equilibrating to room temperature and centrifuging briefly at low rpm.
7. Add 100 µl of Assay Buffer to each sample (in microcentrifuge tubes) and incubate for 5 min at room temperature. For 96-well plates, use 60 µl of Assay Buffer per sample well.
8. Prepare enough of a 1.25 mM CSPD Substrate working dilution for your experiment (see Step 9) by diluting a sufficient volume of the provided 25 mM stock solution 1:20 with Chemiluminescent Enhancer just prior to use (see Section II).

V. Chemiluminescent SEAP Assay continued

9. Add 100 µl of the diluted CSPD Substrate to each sample (in microcentrifuge tubes), and incubate for 10 min at room temperature. For 96-well plates, use 60 µl of the diluted substrate per sample well.
10. The chemiluminescent signal generally remains constant up to 40 min after the addition of the substrate solution. Therefore, readings may be taken between 10–40 min after the addition of CSPD. In order to optimize the assay sensitivity, it is recommended that measurements be performed at various incubation times between 10–40 min in order to determine the point of maximum light emission.

B. Chemiluminescence Detection Methods

1. Detection using a tube luminometer

   If the assay is performed in a tube suitable for luminometer readings the sample may be placed directly in the instrument after Step A.9 and measurements taken following a minimum 10 min incubation. If the assay is not performed in a suitable tube, transfer the entire solution from Step A.9 to the appropriate luminometer tube and place in the instrument. Record light signals as 5 to 15 sec integrals.

2. Detection using a plate luminometer
   a. If the assay was not performed in a luminometer-compatible microtiter plate, transfer the entire solution from each well in Step A.9 to a suitable plate and place it in the instrument.
   b. Record light signals with the manufacturer's recommended luminometer settings.

3. Detection by exposure of x-ray film

   If a luminometer is not available, light emission may be recorded by exposure of x-ray film to opaque white 96-well flat-bottom microtiter plates containing chemiluminescent SEAP assay samples (see Section III). This detection procedure yields spots on the film, which can be quantitated by comparison to positive and negative control incubations.
   a. Perform the entire SEAP assay (Steps A.1–9) in opaque white 96-well flat-bottom microtiter plates.
   b. After at least a 10 min incubation at Step A.9, place the microtiter plate over a piece of x-ray film, cover the plate and film with Saran wrap, and place a heavy object such as a book on top to hold the film in place.
   c. Expose the film for 5–30 min at room temperature.

      Note: For comparisons between samples, it is critical to remain within the linear response range of the x-ray film. In order to avoid misleading results, it is recommended that several different film exposure times be utilized for each microtiter plate.

VI. Fluorescent SEAP Assay

A. Fluorescent SEAP Assay

As noted in Section IV, each construct should be transfected and subsequently assayed in triplicate. It may be necessary to dilute some samples 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 (see Section IV.B).

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

1. Allow a enough Assay Buffer (see Step 7) for performing the entire experiment to equilibrate to room temperature.
2. Prepare the required amount of 1X Dilution Buffer (see Steps 4, 8 & 9) by diluting a sufficient volume of the provided 5X stock solution 1:5 with ddH20 just prior to use (see Section II) and allowing it 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 using a vortex mixer with a plate adaptor.
5. Seal the sample wells of 96-well plates with adhesive aluminum foil (See Section III), and then incubate the diluted samples for 30 min in a 65°C incubator. (If you are performing the assay in microcentrifuge tubes, you can use a heating 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 well and incubate for 5 min at room temperature.
8. Prepare enough of a 1 mM MUP working dilution for your experiment (see Step 9) by diluting the a sufficient volume of the provided 10 mM MUP Fluorescent Substrate 1:10 in 1X Dilution Buffer just prior to use (see Section II).
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.

VI. Fluorescent SEAP Assay continued

B. Fluorescence Detection Methods

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

1. Detection using a plate fluorometer

   If the assays are 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.

2. Detection using a tube fluorometer

   If the assay is performed in a suitable tube for fluorometer readings, the sample may be placed directly in the instrument after Step A.9. Otherwise, transfer the entire solution from Step A.9 to an appropriate tube and place in the instrument.

VII. Troubleshooting Guide

A. Determining the Linear Range of the Assay

If in doubt about the linear range of the assay, prepare and assay a dilution series using the Positive Control Placental Alkaline Phosphatase.

B. Little or No Signal From Transfected Cells

1. Ensure that the assay conditions are correct and that the detection method is working by assaying the positive control enzyme.
2. Ensure that the transfection efficiency has been optimized by using pSEAP2-Control (or a suitable alternative) as an internal positive control for SEAP expression.
3. Increase the number and/or density (or concentration) of cells used in transfections.
4. If background signals from negative controls (i.e., cells transfected with pSEAP2-Basic) are low, increase the volume of media assayed from experimental cultures from 25 µl to 50–75 µl. (The 1X Dilution Buffer added at the next step should be adjusted accordingly.)
5. Increase the post-transfection interval prior to collecting media samples.
6. Ensure that the conditioned media does not contain an inhibitory activity by adding 2 µl of Positive Control Placental Alkaline Phosphatase to 25 µl of culture medium at Step V.A.3 or VI.A.3.
7. If assaying with x-ray film, try increasing the film exposure time.
8. For detection via a tube or plate luminometer, refer to the instrument instructions for methods to increase the sensitivity of light detection.

C. High Background Signals

1. Ensure that all intact cells and cellular debris are removed from the conditioned media by centrifugation in Step IV.D.2. This step is particularly important for suspension cultures.
2. Ensure that the diluted media samples are heated for the full 30 min at 65°C as specified in Step V.A.5 or VI.A.5.
3. The volume of media assayed from experimental cultures (Step V.A.3 or VI.A.3) may be decreased if the signal is sufficiently high. Alternatively, samples may be diluted using 1X Dilution Buffer.
4. If possible, after transfection grow cells in media containing minimal serum. Serum levels >10% (v/v) may increase background.

D. Signal is too High, Exceeding the Linear Range of the Assay

This problem is easily corrected by either assaying a lower volume of conditioned medium at Step V.A.3 or VI.A.3, or diluting the samples using 1X Dilution Buffer.

VII. Troubleshooting Guide continued

E. Recommended Sequencing Primers

We recommend the following primers for sequencing inserts cloned into the pSEAP2 Vectors. (These primers are not available from Clontech.)

To sequence from the TB region into the MCS:

5'-CTAGCAAAATAGGCTGTCCC-3'
(5057–5076 in pSEAP2-Control)

To sequence from the 5' region of the SEAP ORF into the MCS:

5'-CCTCGGCTGCCTCGCGGTTCC-3'
(376–356 in pSEAP2-Control)

To sequence fragments inserted into restriction sites downstream of the SEAP ORF:

5'-GCCTTCGCCGCCTGCCTGGAG-3'
(1708–1728 in the 3' region of the SEAP gene)

VIII. References

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IX. Related Products

For a complete listing of all Clontech products, please visit **www.clontech.com**.

Products	Cat. No.
• Great EscAPe™ SEAP Reporter System 3	631706
• Great EscAPe™ SEAP Chemiluminescence Detection Kit	631701 631702 631725
• Great EscAPe™ SEAP Fluorescence Detection Kit	631704
• pSEAP2-Basic Vector	631715
• pSEAP2-Control Vector	631717
• Fluorescent Protein Vectors	many
• Luminescent β-gal Reporter System 3	631713
• Luminescent β-gal Detection Kit II	631712
• CalPhos™ Mammalian Transfection Kit	631312
• Clonfectin™	631301
• Pathway Profiling SEAP System	631910
• Pathway Profiling SEAP System 2	631020
• pAP1(PMA)-SEAP Vector	631907
• pAP1-SEAP Vector	631903
• pSRE-SEAP Vector	631901
• pGRE-SEAP Vector	631902
• pNFkB-SEAP Vector	631905
• pCMV-β Vector	631719

Appendix A: Plasmid Maps & Multiple Cloning Sites

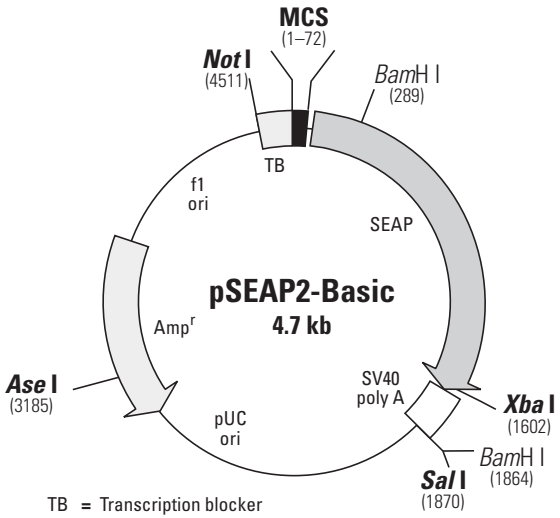
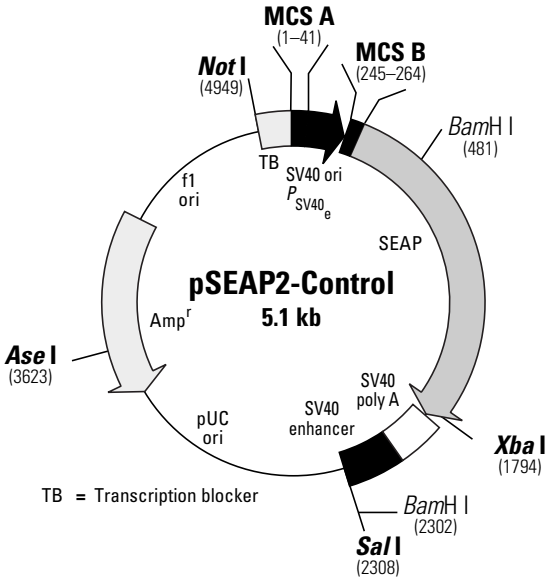


Figure 2. Map and multiple cloning site of pSEAP2-Basic. pSEAP2-Basic lacks eukaryotic promoter and enhancer sequences. The MCS allows promoter DNA fragments to be inserted upstream of the SEAP gene. Enhancers can be cloned into either the MCS or unique downstream sites. Unique restriction sites are in bold.

The Great EscAPe vectors contain the SV40 late polyadenylation signal inserted downstream of the SEAP coding sequences to ensure proper and efficient processing of the transcript in eukaryotic cells. A synthetic transcription blocker (TB), composed of adjacent polyadenylation and transcription pause sites, reduces background transcription (Eggermont & Proudfoot, 1993). The vector backbone contains an f1 origin for single-stranded DNA production and a pUC19 origin of replication and an ampicillin resistance gene for propagation in E. coli. The multiple cloning site region is identical in both pSEAP2-Basic & pSEAP2-Control Vectors except for a 209 bp promoter fragment that has been inserted between the Bgl II and Hind III sites in pSEAP2-Control.

The complete sequence information for the Great EscAPe SEAP2 Vectors can be downloaded from our web site at **www.clontech.com**.

Appendix A: Plasmid Maps & MCSs continued



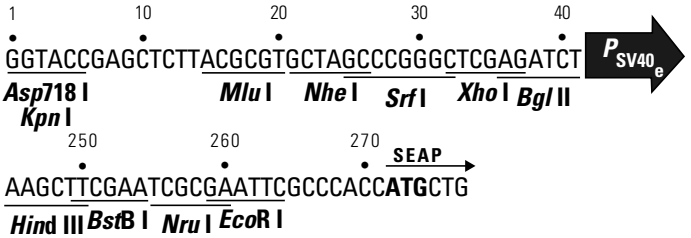


Figure 3. Map and multiple cloning site of pSEAP2-Control. pSEAP2-Control is pSEAP2-Basic with the SV40 early promoter inserted upstream of the SEAP gene and the SV40 enhancer inserted downstream. pSEAP2-Control expresses SEAP in most cell types and provides an important positive control in most experiments. Unique restriction sites are in bold.

See the description of pSEAP2-Basic (Figure 2) for information about elements common to both Great EscAPe vectors.

The complete sequence information for the Great EscAPe SEAP2 Vectors can be downloaded from our web site at **www.clontech.com**.

Notes

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