ab133077 – SEAP Reporter Gene Assay Kit (Luminescence)

Instructions for Use

For the sensitive quantitation of SEAP in conditioned cell culture medium from transfected cells.

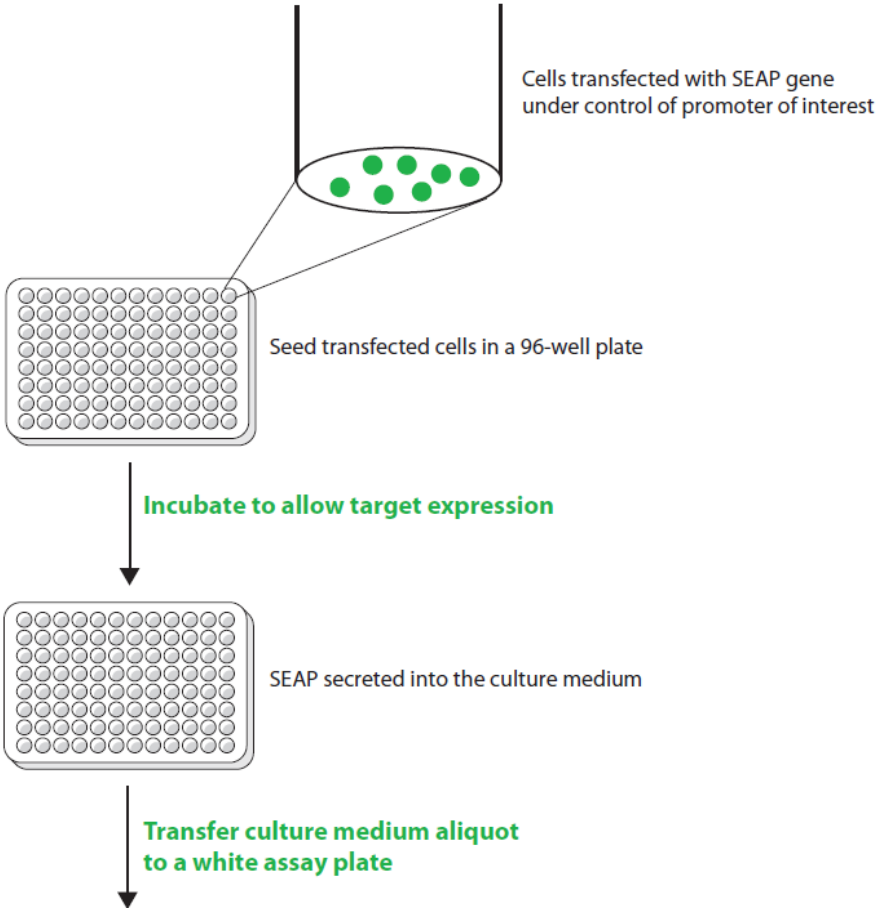
View kit datasheet: www.abcam.com/ab133077
(use www.abcam.cn/ab133077 for China, or www.abcam.co.jp/ab133077 for Japan)

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

ab133077 provides a simple chemiluminescence method for the sensitive quantitation of SEAP in conditioned cell culture medium from transfected cells. The assay can detect SEAP activity in the microunits/well (mU/ml) range. The kit includes enough reagents to run three 96-well plates. The assay is easy to perform and can be completed within one hour.


Cells transfected with SEAP gene
under control of promoter of interest
Seed transfected cells in a 96-well plate
Incubate to allow target expression
SEAP secreted into the culture medium
Transfer culture medium aliquot
to a white assay plate

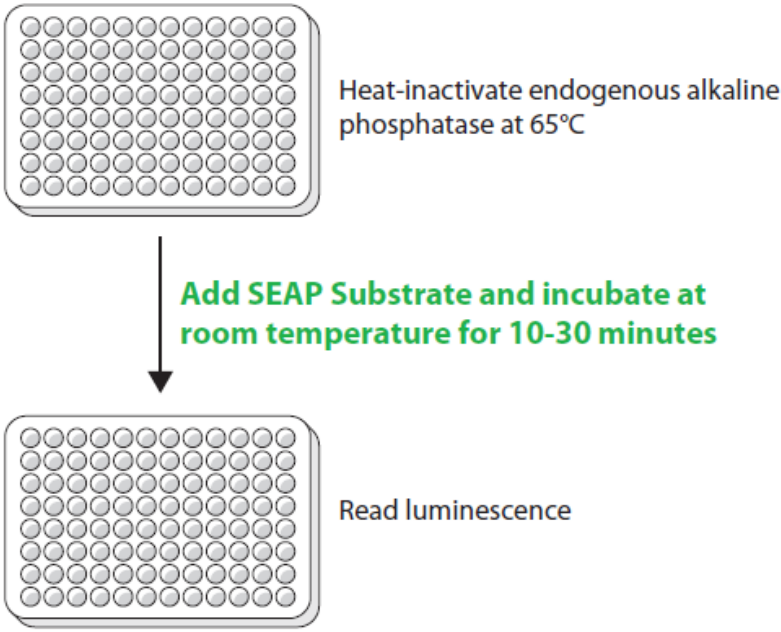


Figure 1. Flow diagram for expression and detection of a SEAP Reporter using a chemiluminescence substrate.

2. Background

Secreted alkaline phosphatase (SEAP) is commonly used as a reporter of gene expression. Compared to other conventional intracellular reporters such as chloramphenicol acetyltransferase (CAT) and firefly luciferase, SEAP has the advantage of being secreted from transfected cells into the culture medium. SEAP activity in the culture medium is directly proportional to changes in intracellular concentrations of SEAP mRNA and protein. In addition, the kinetics of gene expression can be studied using the same cultures by repeatedly collecting culture medium at different time points. The intact cells can be used for further analysis of RNA or protein expression.

SEAP activity was first measured using the chromogenic alkaline phosphatase substrate *p*-nitrophenyl phosphate (*p*NPP). Today, the most sensitive SEAP assays employ chemiluminescent alkaline phosphatase substrates such as the 1,2-dioxetane CSPD. Chemiluminescent detection of SEAP is fast, easy to perform, and sensitive (see Figure 1).

3. Components and Storage

For best results, remove components and store as stated.

Item	Quantity	Storage
SEAP Substrate (Luminescence)	15 ml	4°C
Cell-Based Alkaline Phosphatase Standard	200 µl	4°C
96-Well Solid Plate (white) with lid	3 plates	RT

Materials Needed But Not Supplied

- A plate reader with capacity to measure chemiluminescence.
- Adjustable pipettes and a repeat pipettor.
- A source of pure water; glass distilled or HPLC-grade water is acceptable.
- A 65°C incubator.

4. Pre-Assay Preparation

A. Cell Culture Preparation

1. Transfect cells with a promoter construct driving the expression of SEAP. Mock-transfected cells should be included as a control.

2. Culture the cells in a CO_2 incubator at 37°C for 24-48 hours, or for the period of time used in your typical experimental protocol.

3. Remove culture medium to measure SEAP activity by following the procedure described in Assay Protocol.

5. Assay Protocol

A. Preparation of Assay Specific Reagents

Cell-Based Alkaline Phosphatase Standard

NOTE: Use the same culture medium, such as MEM or DMEM, as you are using for your cells to prepare the Cell-Based Alkaline Phosphatase Standard. If you are using culture medium containing FBS to prepare the Cell-Based Alkaline Phosphatase Standard, inactivate endogenous alkaline phosphatase by heating the medium at 65°C for 30 minutes before use.

To prepare the standard for use in the SEAP Assay:

1. Obtain eight clean test tubes and label them #1 through #8.

2. Aliquot 990 µl of Culture Medium into tube #1 and 100 µl into tubes #2 - #8.

3. Transfer 10 µl of the Cell-Based Alkaline Phosphatase Standard into tube #1 and mix thoroughly. The activity of this Cell-Based Alkaline Phosphatase Standard, the first point on the standard curve, is 50 mU/ml.

4. Serially dilute the standard by removing 100 µl from tube #1 and place into tube #2; mix thoroughly.

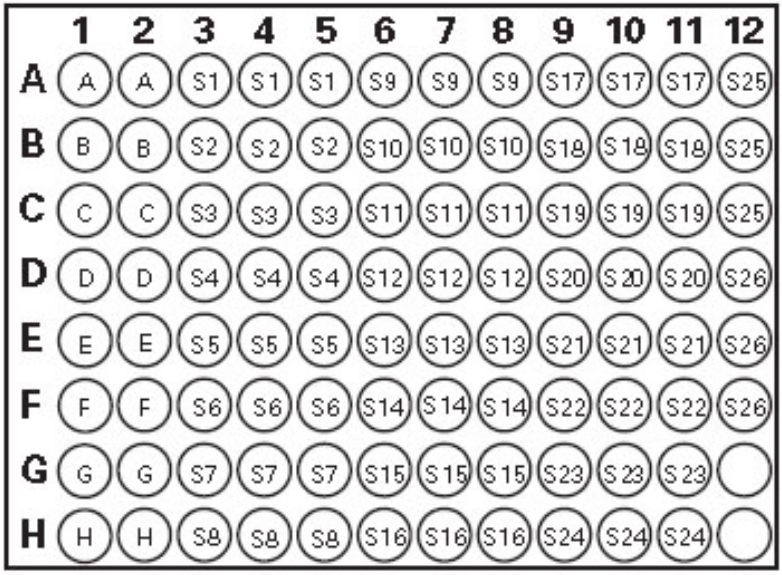
5. Next, remove 100 µl from tube #2 and place into tube #3; mix thoroughly.

6. Repeat this procedure for tube #4 to tube #7.

7. Do not add any standard to tube #8. This tube will be the blank.

4. Plate Set Up

Each plate should contain a standard curve and wells containing conditioned cell culture medium from mock-transfected cells or transfected cells. We recommend that standards be run in duplicate and that each treatment be performed in triplicate. A suggested plate format is shown below in Figure 2. The user may vary the location and type of wells present as necessary for each particular experiment.



A-H – Standards S1-S26 – Sample Wells

Figure 2. Plate Format

5. Performing the Assay

Pipetting Hints:

- *Use different tips to pipette each reagent.*
- *Before pipetting each reagent, equilibrate the pipette tip in that reagent (i.e., slowly fill the tip and gently expel the contents, repeat several times.*
- *Do not expose the pipette tip to the reagent(s) already in the well.*

Prior to use in the assay, remove the SEAP Substrate from the refrigerator and allow to equilibrate to room temperature.

1. In a tissue culture hood, transfer 10 µl of culture medium from each well to the corresponding well of a 96-Well Solid Plate (white) with lid. Cover the plate with the lid.

2. Inactivate endogenous alkaline phosphatase by heating the samples at 65°C for 30 minutes. The SEAP expressed in this assay is stable under these conditions.

3. Remove the plate from the incubator and allow to equilibrate to room temperature.

4. Add 10 µl of standards prepared above to the corresponding wells of the white plate.

5. Add 50 µl of substrate to each well and shake briefly. Incubate 10-30 minutes.

6. Read the plate with a plate reader capable of detecting chemiluminescence.

6. Data Analysis

A. Plot the Standard Curve

Make a plot of Relative Luminescence Units (RLU) as a function of alkaline phosphatase activity and determine the equation of the line. See Figure 3 for a typical standard curve.

B. Determine the Sample Concentration

If you anticipate a high production of SEAP after transfection and you are planning to use the Cell-Based Alkaline Phosphatase Standard to calculate the level of SEAP production, dilution of your samples may be required to obtain values that fall on the standard curve.

C. Determination of SEAP Activity

SEAP Activity (mU/ml) = [RLU - (y-intercept)]/Slope

D. Performance Characteristics

The standard curve presented here is an example of the data typically produced with the assay. However, your results will not be identical to these. You must run a new standard curve for each experiment.

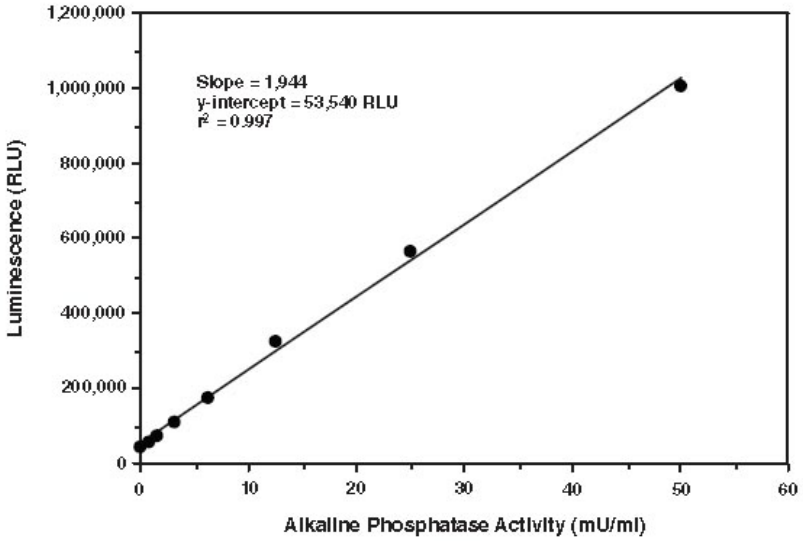


Figure 3. Cell-Based Alkaline Phosphatase Standard curve.

7. Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates	A. Poor pipetting technique. B. Bubble in well(s).	A. Be careful not to splash the contents of the wells. B. Carefully tap the side of the plate with your finger to remove bubbles.
No SEAP activity was detected in the sample	Cells were not transfected.	Optimize your transfection protocol.
No alkaline phosphatase activity in the standards	The standards are degraded.	Prepare fresh standards.

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