Secreted Alkaline Phosphatase Reporter Gene Assay Kit (Luminescence)

Item No. 600260

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a 4°C kit. For best results, remove components and store as stated below.

Item Number	Item	Quantity/Size	Storage
600183	SEAP Substrate (Luminescence)	1 vial/15 ml	4°C
600261	Cell-Based Alkaline Phosphatase Standard	1 vial/200 µl	4°C
700029	96-Well Solid Plate (white)	3 plates	RT
400012	96-Well Cover Sheet	3 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone:	888-526-5351 (USA and Canada only) or 734-975-3888
Fax:	734-971-3641
Email:	techserv@caymanchem.com
Hours:	M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section on page 3 and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

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

INTRODUCTION

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.[1] 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).[2] 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, on page 6).

About This Assay

Cayman's Secreted Alkaline Phosphatase Reporter Gene Assay Kit (Luminescence) 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 milliunits/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.

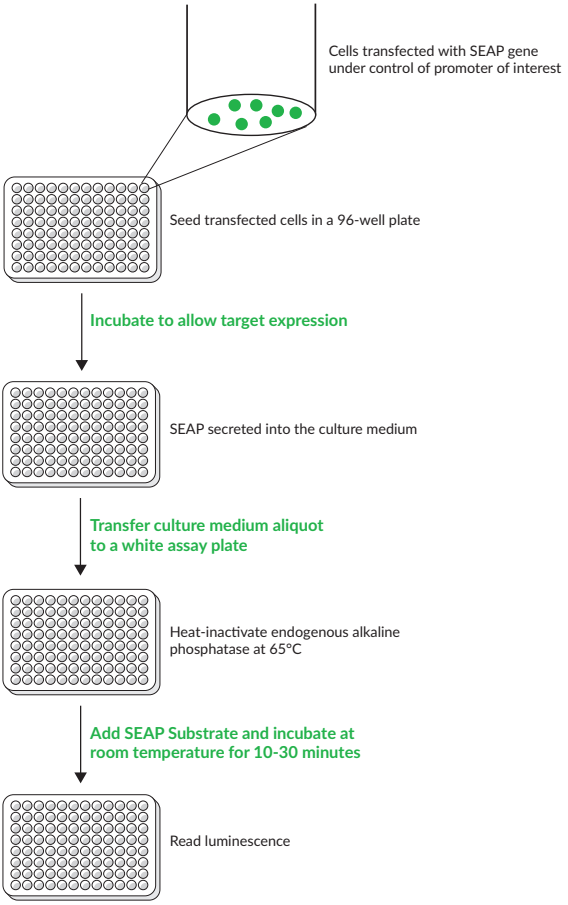


Figure 1. Flow diagram for expression and detection of a SEAP Reporter using a chemiluminescence substrate

PRE-ASSAY PREPARATION

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 the **Assay Protocol**, on page 10.

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: Obtain eight clean test tubes and label them #1 through #8. Aliquot 990 µl of Culture Medium into tube #1 and 100 µl into tubes #2-#8. Transfer 10 µl of the Cell-Based Alkaline Phosphatase Standard (Item No. 600261) 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. Serially dilute the standard by removing 100 µl from tube #1 and place into tube #2; mix thoroughly. Next, remove 100 µl from tube #2 and place into tube #3; mix thoroughly. Repeat this procedure for tube #4 to tube #7. Do not add any standard to tube #8. This tube will be your blank.

NOTE: 1 Unit (U) is the amount of enzyme causing the hydrolysis of 1 µmol of p-Nitrophenyl phosphate per minute at pH 9.6 and 25°C (glycine buffer).

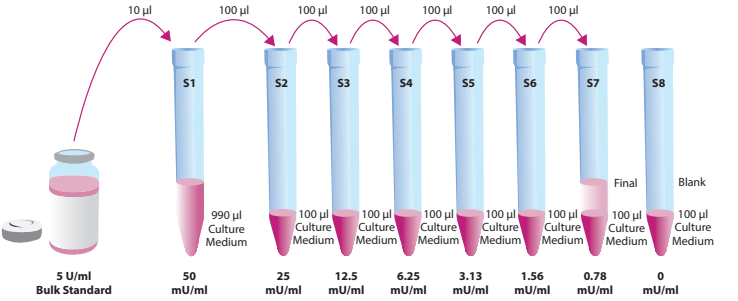
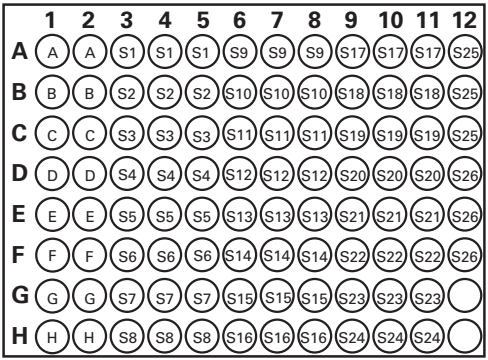


Figure 2. Preparation of the Cell-Based Alkaline Phosphatase Standards

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 3. The user may vary the location and type of wells present as necessary for each particular experiment. We suggest that you record the contents of each well on the template sheet provided (see page 14).



A-H = Standards
S1-S26 = Sample Wells

Figure 3. Sample plate format

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) Item No. 700029). Cover the assay plate with the 96-Well Cover Sheet (Item No. 400012).
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

ANALYSIS

Calculations

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 4, on page 12, for a typical standard curve.

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.

Determination of SEAP Activity

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

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.

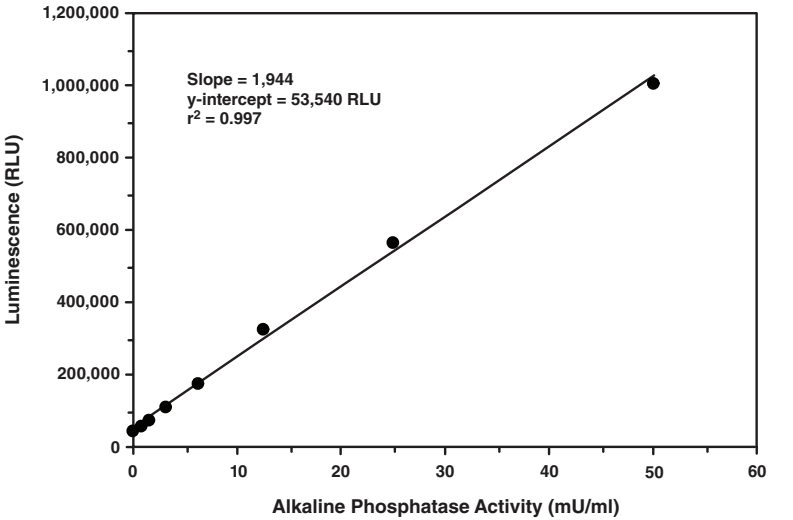


Figure 4. Cell-Based Alkaline Phosphatase Standard curve

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates	A. Poor pipetting/technique B. Bubble in the 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

References

1. de Lecea, L., Kilduff, T.S., Peyron, C., *et al. Proc. Natl. Acad. Sci. USA* **95**, 322-327 (1998).
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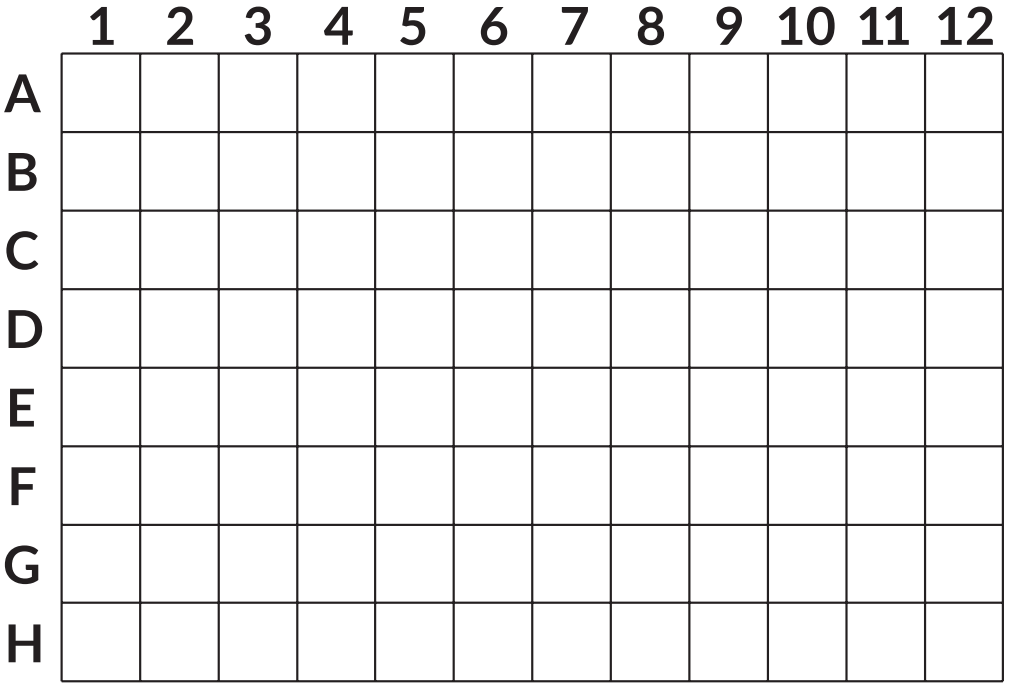
1 2 3 4 5 6 7 8 9 10 11 12
A
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NOTES

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