Data sheet

SEAP Reporter Gene Assay Kit
(Luminescence)

Cat. No: CA040
3 x 96 well plate

Introduction

Secreted alkaline phosphatase (SEAP) is commonly used as a reporter of gene expression. 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. 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. 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.

SEAP assays utilize enzyme activity of alkaline phosphatase to dephosphorylate the chemiluminescent alkaline phosphatase substrate CSPD into an unstable dioxetane anion which decomposes and emits light.

Advantages/Features:

- Cell lysis is not required for analysis so a single set of cells can be used for both the SEAP assay and another purpose.
- Preparation of cell lysates is not required for analysis.
- Rapid results within 1 hour.
- Sensitivity can be assayed even in low cell concentrations.
- The entire assay can be performed directly in a microtiter plate.

Kit Contents

Component	Amount	Store
96W Solid Plate (white) with lid	3 units	RT
Alkaline Phosphatase Standard	1vial x 50µl	4ºC
SEAP Substrate (Luminescence)	1vial x 15ml	4ºC

Storage

Upon receipt, store the kit at +4ºC. Component solution containing CSPD® substrate Store at 2-6°C, placental alkaline phosphatase positive control Store at ≤ -20°C Protect SEAP Substrate from light. Kit components are stable for at least 12 months from date of receipt when stored as recommended.

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Assay Procedure

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

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 "B. Assay Protocol".

B. Assay Protocol

1. Transfer 10 µl of culture medium from each well to the corresponding well of a 96-Well Solid Plate (white) with lid.
2. Add 10 µl of culture medium in a control well as a blank.
3. Cover the plate with the lid.
4. 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.
5. Remove the plate from the incubator and allow to equilibrate to room temperature.
6. Add 10 µl of standards prepared above to the corresponding wells of the white plate.
   If you are using culture medium containing FBS to prepare the Alkaline Phosphatase Standard, inactivate endogenous alkaline phosphatase by heating the medium at 65°C for 30 minutes before use.
7. Add 50 µl of substrate to each well and shake briefly. Incubate 10-60 minutes.
8. Read the plate with a plate reader capable of detecting chemiluminescence.

C. Plot the Standard Curve

D. Determine the Sample Concentration

E. Determination of SEAP Activity

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

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to **www.canvaxbiotech.com** for Material Safety Data Sheet of the product.