

DATA SHEET

Version: 03
Revision date: 28/03/2023Tlf: +34 983 54 85 63
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1. Identification

Product name **SEAP Reporter Gene Assay Kit**
288 assays (3 x 96 well plate)

Cat. No: **CA040**

2. Description

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.

3. Composition & Storage

Item	Quantity	Storage
96W Solid Plate (white) with lid	3 units	RT
Adhesive strips	3 units	RT
Alkaline Phosphatase Standard	1vial x 50 µl	-20°C
SEAP Substrate (Luminescence)	1vial x 15 µl	4°C

4. 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.

5. Applications

Measurement the levels of SEAP in the culture medium of transfected cells.

6. 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 CO₂ 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".



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B. Assay Protocol

- Transfer 10 µl of culture medium from each well to the corresponding well of a 96-Well Solid Plate (white) with lid.
- Add 10 µl of culture medium in a control well as a blank.
- Cover the plate with the lid.
- 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.
- Remove the plate from the incubator and allow to equilibrate to room temperature.
- 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.
- Add 50 µl of substrate to each well and shake briefly. Incubate 10-60 minutes.
- 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

$$\text{SEAP Activity (mU/ml)} = [\text{IRLU} - (\text{y-intercept})] / \text{Slop}$$

7. Further information

Product Use Limitations	This product is developed, designed and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
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