



PRODUCT INFORMATION & MANUAL

Secreted Alkaline Phosphatase Reporter (SEAPorter™)
Assay Kit

NBP2-25285

Research use only. Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 888.506.6887 - technical@novusbio.com

Novus kits are guaranteed for 6 months from date of receipt.

TABLE OF CONTENTS

I. Introduction	3
II. Kit Description and Advantages	4
III. Kit Components and Storage.....	5
IV. Protocol	6
V. Measurement of SEAP	6
VI. References	8
VII. Trouble Shooting.....	8
VIII. Related Products.....	8

I. INTRODUCTION

Placental alkaline phosphatase (PLAP) is one of the most stable isoenzymes, only existing in the placenta of higher primates. These characteristics make placental alkaline phosphatase suitable to use as a reporter gene for the analysis of promoter activity and gene expression in cell culture and animal serum. The natural form of PLAP is membrane anchored. The recombinant form of placental alkaline phosphatase (secreted alkaline phosphatase, SEAP) is used for reporter gene function. SEAP is designed by inserting a translational terminator after amino acid 489 (Berger, et al., *Gene* 66 (1): 10 (1988)). This mutation converts the membrane-bound PLAP protein into the secreted protein SEAP.

SEAP Reporter Advantages:

- Sampling Ease: SEAP is secreted into the media, so no cell lysis is required for the detection of its enzymatic activity.
- A secreted indicator function permits multiple kinetics experiments using only one culture through sequential sampling of the medium and allows the cells to be used for other purposes, such as RNA extraction, western blot analysis, and other assays.
- Heating samples at 65°C for 10-30 mins can destroy endogenous alkaline phosphatase but not SEAP.
- SEAP is stable in serum, allowing it to be used as a reporter assay *in vivo*.
- SEAP catalyzes the hydrolysis of pNitrophenyl phosphate (PNPP) producing a yellow product that can be read in a spectrophotometer or ELISA reader at 405 nm.

II. KIT DESCRIPTION

This Kit provides sufficient reagents for 400 quantitative measurements of SEAP protein in 96-well microtiter format. All 96 wells can be used at one time or you may use only the wells as needed by your experimental design. Use of duplicate wells for the SEAP standard is recommended to obtain accurate results. This kit can be used with supernatant from transfected cells as described or serum as determined by your experimental protocol.

The kit advantages include:

- Multiple samples can be analyzed in a low-volume, high throughput experiment.
- Full analysis complete in under 1 hr.
- Quantitative nature of assay allows direct measurement of SEAP protein in cell supernatant and serum.

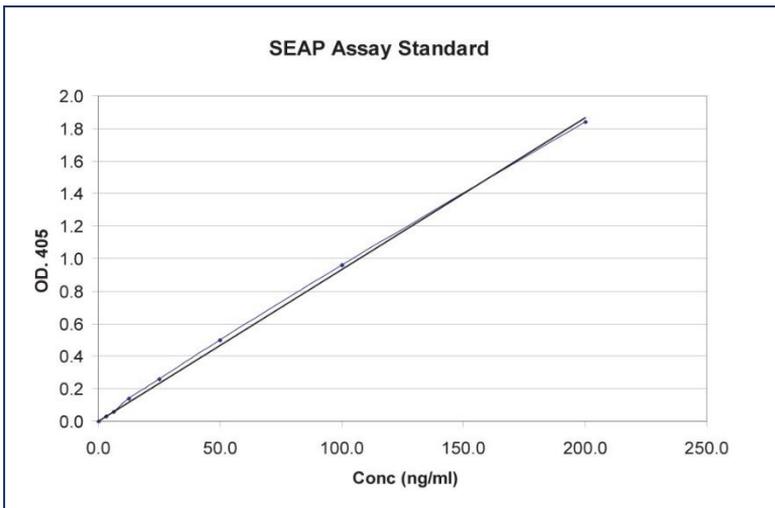


Figure 1: Standard curve for the SEAP protein provided in the SEAPorter™ Assay Kit (NBP2-25285). A serial dilution of SEAP protein was added to wells of a 96-microtiter plate and the standard curve was generated according to the Kit assay protocol.

III. KIT COMPONENTS AND STORAGE

The components included in this kit need to be stored at the specified temperatures:

Part No.	Kit Components	Quantity	Storage
IMK-515-1	Secreted Placental Alkaline Phosphatase standard, SEAP (200 µg/mL)	10 µL	-20°C
KC-103B	PNPP Substrate	8 tablets (5 mg each)	-20°C
IMK-515-6	Sample Dilution Buffer (10X)	10 mL	4°C
IMK-515-7	PNPP Buffer (10X)	4 mL	4°C
N/A	96 well Assay Plates	4 EA	RT
N/A	Plate Sealer	4 EA	RT

Additional materials needed but not provided in the kit:

- Centrifuge
- 37°C CO₂ incubator
- 65°C water bath or heating block
- ELISA plate reader
- Multichannel pipette
- Lipid for transfection

IV. PROTOCOL

Reagents Preparation

Note: The protocol has been optimized at Novus using the buffers and reagent in the kit. Substitution with other reagents may not give optimal results. Reagents are prepared at room temp. just prior to the Assay.

Note: The supernatant can be used immediately or stored at -70 °C for later use.

SEAP Standard Stock Solution (400 ng/mL):

The SEAP protein standard is supplied at 200 µg/mL. Prepare stock solution by diluting 1 µL of SEAP protein to 499 µL of dilution buffer. Diluted Standard can be stored at -20°C for future use. An excessive amount of SEAP standard is provided to maximize test size flexibility.

PNPP Substrate:

1. PNPP buffer (1X): Dilute 10X PNPP buffer with sterile, H₂O (e.g. 1 mL to 9 mL sterile H₂O).
2. Dissolve one 5 mg PNPP substrate tablet in 5 mL of 1X PNPP Buffer (giving a final concentration of 1 mg/mL). Prepare just before use.

Sample Dilution Buffer:

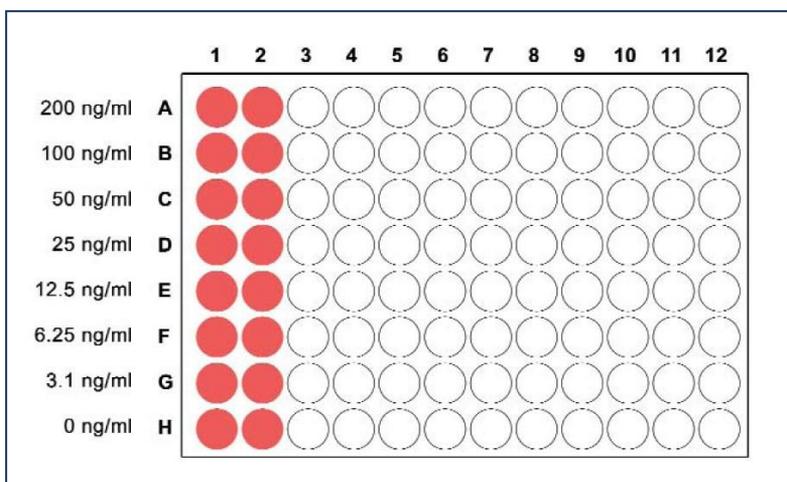
Dilute 10X Dilution Buffer to 1X with sterile H₂O (e.g. 1 mL of 10X dilution buffer to 9 mL of sterile H₂O).

V. MEASUREMENT OF SEAP

This kit allows quantitative measurements of SEAP protein in 96-well microtiter formats. All 96 wells can be used at one time or you may use only the wells as required by your experimental design. Use of duplicate wells for each time point or control is recommended to obtain accurate results. This kit can be used for transfected cell supernatant or serum.

1. SEAP standard: Label eight Eppendorf tubes from A-H. Add 50 µL of Dilution Buffer to tubes A-H. Add 50 µL of 400 ng/mL SEAP standard to tube A. Mix well by pipetting up and down a few times. Take 50 µL from tube A and transfer it to tube B. Continue this serial dilution to tube G. Tube H only contains 50 µL of Dilution Buffer. This will serve as a blank for generating the standard curve. The remaining diluted standard can be stored at -20°C for future use (aliquot to minimize freeze - thaw cycle).

2. Samples: Transfected samples can be diluted 1:2 or 1:10 in Dilution Buffer depending on the efficiency of transfection.
3. Load samples and standard to microtiter plate: Add 10 μL of diluted SEAP standard to columns 1 and 2 of the microtiter plate. Add 10 μL of diluted samples to the plate. Add 10 μL of H_2O to each well containing a sample or standard.
4. Seal the microtiter plate with plate sealer and incubate at 65°C for 30 minutes to inactivate any endogenous alkaline phosphatase and allow for precise quantification of SEAP.
5. Spin plate briefly in a centrifuge to return all liquid to the bottom of the well. If this equipment is not available, gently tap the sealed plate on a hard surface until all the liquid is down.
6. Add 100 μL of the 1 mg/mL PNPP substrate solution to each well. Incubate at room temp.
7. Take absorbance readings at 405 nm after 30 minutes and 1 hr in ELISA plate reader.



VII. REFERENCES

1. Cullen B.R. and Malim M.H, Method Enzymol. 216: 362-368 (1992).
2. Berger J and Cullen B.R, Gene 66: 1-10 (1988).

VII. TROUBLE SHOOTING

Problem	Possible Cause	Suggestion
Weak or no SEAP expression in the supernatant	Poor transfection efficiency	Use pCMV/SEAP plasmid to check transfection efficiency
	Expired substrate	Use freshly prepared substrate
	Insufficient incubation time	Incubate longer

VIII. RELATED PRODUCTS

Plasmids

Part Number	Description
IMK-515-3	pNF-kB/SEAP plasmid
IMK-515-4	pCMV/SEAP plasmid

Antibodies

For a complete list of antibodies against TLR and NF-kB signaling pathway, please visit our website www.novusbio.com