

Assay kits

SEAP Assay Kit

SEAP - Secreted Alkaline Phosphatase Assay Kit

Protocol

IMPORTANT NOTES – Before you begin

Fast and easy, accurate staining kit for the measurement of Secreted Embryonic Alkaline Phosphatase activity.

- ✓ The SEAP – Secreted Alkaline Phosphatase assay Kit is a colorimetric assay for sensitive quantification of SEAP in culture medium from transfected cells. Alkaline phosphatase cleaves the colourless substrate 4-nitrophenylphosphate (pNPP) forming the yellow product p-nitrophenolate in alkaline solutions (figure 1).

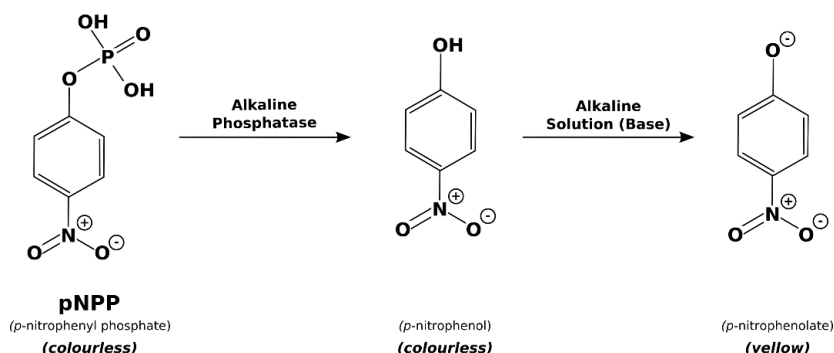



Figure 1: Secreted alkaline phosphatase catalyses the hydrolysis of pNitrophenyl phosphate (pNPP) producing a yellow product under alkaline conditions and can be conveniently measured at 405 nm on a spectrophotometer or an ELISA reader.

- ✓ Secreted Alkaline Phosphatase (SEAP) is commonly used as a reporter gene expression. It is a truncated form of placental alkaline phosphatase (GPI-anchored protein). The SEAP enzyme is secreted in the culture medium which allows the assay to be performed on small samples of cell supernatants. In that way, transfected cells are not hampered by the read out procedure and the same cultures can be repeatedly used for kinetics experiments. The SEAP enzyme presents an extreme heat stability and resistance to phosphatase inhibitor L-homoarginine. Endogenous alkaline phosphatase activity in transfected cells can be eliminated by pretreatment of sample at 65°C.


The level of SEAP activity detected in the culture medium is directly proportional to changes in intracellular concentration of SEAP mRNA and proteins ¹.

- Transfected cells remain intact: no need to process samples.
- No preparation of cells is required (lysate, fixation...): faster & more convenient than assaying intracellular reporter.
- The protein is secreted in supernatants: multiple samples can be analysed in low volumes.
- Samples can be used immediately or stored at -20°C for later use, or -80°C for longer storage

For additional information and protocols (optimization, scaling, co-transfection...) tips, troubleshooting or other applications

 www.ozbiosciences.com

Any questions?

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SEAP – Secreted Alkaline Phosphatase Assay Kit | Specifications

Package content	SP00500 pNPP-containing Assay Buffer (component A): 10 x 1 mL Reaction Buffer #1 (component B): 125 mL Reaction Buffer #2 (component C): 125 µL Dilution Buffer (component D): 20 mL
Shipping conditions	The kit is shipped at room temperature.
Storage conditions	Upon receipt and for long-term use, store all reagent tubes at the indicated storage conditions pNPP-containing Assay Buffer (component A): -20°C Reaction Buffer #1 (component B): +4°C Reaction Buffer #2 (component C): +4°C Dilution Buffer (component D): +4°C
Shelf life	1 year from the date of purchase when properly stored and handled
Important notice	For research use only. Not for use in diagnostic procedures.

1. Pre-Assay preparation

For *in vitro* transfection:

1. Plate the cells 24H before transfection experiment in complete medium.
2. Transfect the cells using Lipofection (such as DreamFect™ Gold) or Magnetofection™ (such as PolyMag Neo or Magnetofectamine™) transfection reagents and SEAP encoding DNA plasmid (pVectOZ-SEAP)*.
3. Culture the cells in CO₂ incubator at 37°C.
4. Post-transfection (from 8 to 96 hours) take a sample of supernatants and transfer to a 96-well plate for assaying.
5. Perform the SEAP – Secreted Alkaline Phosphatase assay Kit according to the protocol below.

* for a complete list of transfection reagents, please refer to <http://www.ozbiosciences.com>

For *in vivo* transfection or infection

1. Proceed to targeted *in vivo* gene delivery using non-viral (*in vivo* DogtorMag™ or *in vivo* PolyMag™) or viral (*in vivo* ViroMag™) MagnetoFection™ method**.
2. Withdraw blood and collect Serum.
3. Take a sample of serum.
4. Perform the SEAP – Secreted Alkaline Phosphatase assay Kit according to the protocol below.

** for more information on *in vivo* transfection reagents, please refer to <http://www.ozbiosciences.com>

2. Working solution preparation

The following protocol described the SEAP assay kit performed in a 96-well plate.

For a full 96-well plate experiment, prepare 20 mL of the working solution as described below. For other assays formats, refer to the table 1 below.

- Thaw pNPP-containing Assay Buffer (component A) and allow all the components to reach room temperature before use.
- Mix 2 mL of component A with 18 mL of Reaction Buffer #1 (component B).
- Add 20 µL of Reaction Buffer #2 (component C) to the previous mix.

Resulting solution should have a light-yellow color. Avoid repeated freeze/thaw cycles.

NOTE: An alkaline phosphatase (AP) standard (EIA grade calf intestine alkaline phosphatase), not provided in this kit, can be used to generate a standard curve from 1 to 100 pg per well.

Table 1: suggested volumes depending on the number of assays in a 96 well plate.

Number of samples	Component A	Component B	Component C
10 wells	0.2 mL	1.8 mL	2 μ L
25 wells	0.5 mL	4.5 mL	5 μ L
50 wells	1.0 mL	9.0 mL	10 μ L
75 wells	1.5 mL	13.5 mL	15 μ L
100 wells (96 well-plate)	2.0 mL	18 mL	20 μ L
1 well ¹	0.02 mL	0.18 mL	0.2 μ L

¹ indicative number: multiply each component values depending on the number of assays to perform.

3. General Protocol

Sample preparation

1. Transfer a sample of culture medium (supernatant) from each well of transfected cells to a 1.5 mL tube.

NOTE: for suspension cells, centrifuge the cells 5 min x 900 rpm before taking a sample of culture medium.

2. Heat the samples 30 minutes at 65 °C to inactivate endogenous alkaline phosphatase.
3. Briefly centrifuge the tubes.
4. Equilibrate the samples to room temperature (optional: cool the samples on ice for 2-3 min).
5. Transfer 20 μ L of sample to the corresponding wells of a 96-well plate.
6. Add 10 μ L per well of Dilution Buffer (component D).

Performing the assay

1. Add 200 μ L per well of the Working solution (refer to 2.2).
2. Incubate 5 to 45 min at room temperature (depends on SEAP level and activity)
3. Measure the absorbance of the converted dye on a plate reader at 405 nm.

NOTE: SEAP stability allows performing experiment kinetics, transfer an aliquot of the supernatants under sterile conditions and return the cell plate into the incubator for further tests.

4. Performance characteristics

This kit proposes a high detection range to measure placental alkaline phosphatase with minimal background. Please refer to the results for more data

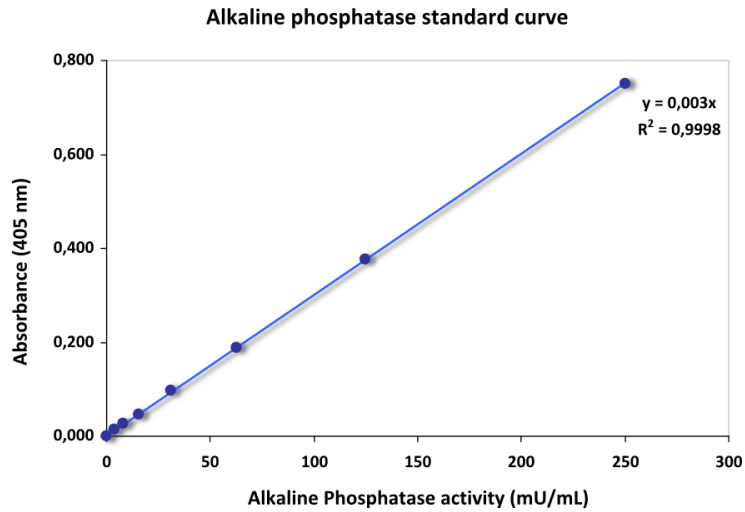


Figure 2: Alkaline phosphatase standard curve. A serial two-fold dilution of alkaline phosphatase standard was prepared and the absorbance was measured at 405 nm.

Additional products

- **OZBlue Cell Viability kit** for cell viability measurement
- **ROS Detection Assay Kit** for measuring Reactive Oxygen Species activity within your cells

Purchaser Notification

Limited License

The purchase of the SEAP -Secreted Alkaline Phosphatase Assay Kit grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in this protocol). This reagent is intended for in-house research only by the buyer. Such use is limited to the transfection of nucleic acids as described in the product manual. In addition, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences. Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of the SEAP -Secreted Alkaline Phosphatase Assay Kit. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact us at OZ Biosciences. Buyers may end this License at any time by returning all SEAP -Secreted Alkaline Phosphatase Assay Kit reagents and documentation to OZ Biosciences, or by destroying all D-Luciferin components. Purchasers are advised to contact OZ Biosciences with the notification that a SEAP -Secreted Alkaline Phosphatase Assay Kit is being returned in order to be reimbursed and/or to definitely terminate a license for internal research use only granted through the purchase of the kit(s). This document covers entirely the terms of the SEAP -Secreted Alkaline Phosphatase Assay Kit research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.

Product Use Limitations

SEAP -Secreted Alkaline Phosphatase Assay Kit and all of its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the use of the kit components by following proper research laboratory practices.

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