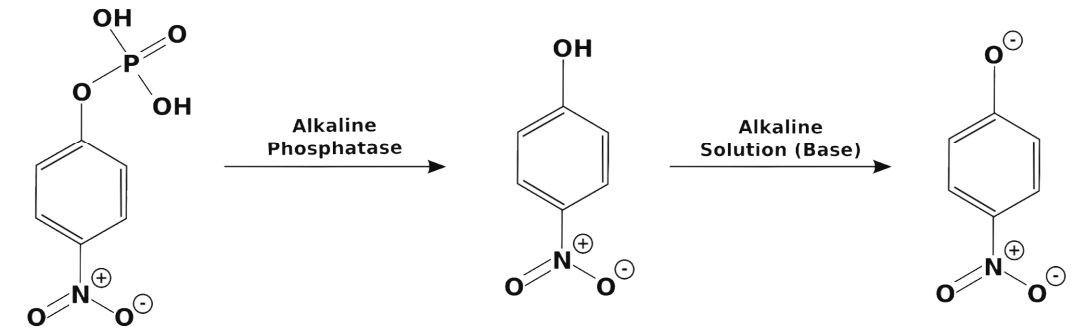
SEAP Assay Kit

INSTRUCTION MANUAL



pNPP
(*p*-nitrophenyl phosphate)
(colourless)

(*p*-nitrophenol)
(colourless)

(*p*-nitrophenolate)
(yellow)

SEAP – Secreted Alkaline Phosphatase Assay Kit

Instruction Manual

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

Catalog Number: SP00500

You can order this product by contacting us. For all other additional information, do not hesitate to contact our dedicated technical support (tech@ozbiosciences.com).

OZ Biosciences SAS
163 avenue de Luminy
Case 922, zone entreprise
13288 Marseille cedex 09 - FRANCE
Ph: +33 (0) 486 948 516
Fax: +33 (0) 486 948 515
contact@ozbiosciences.com
order@ozbiosciences.com

OZ Biosciences INC
4901 Morena Blvd,
Suite 501
San Diego CA 92117 - USA
Ph : + 1-858-246-7840
Fax : + 1-855-631-0626
contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com

www.ozbiosciences.com

1. Technology

1.1. Description

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).

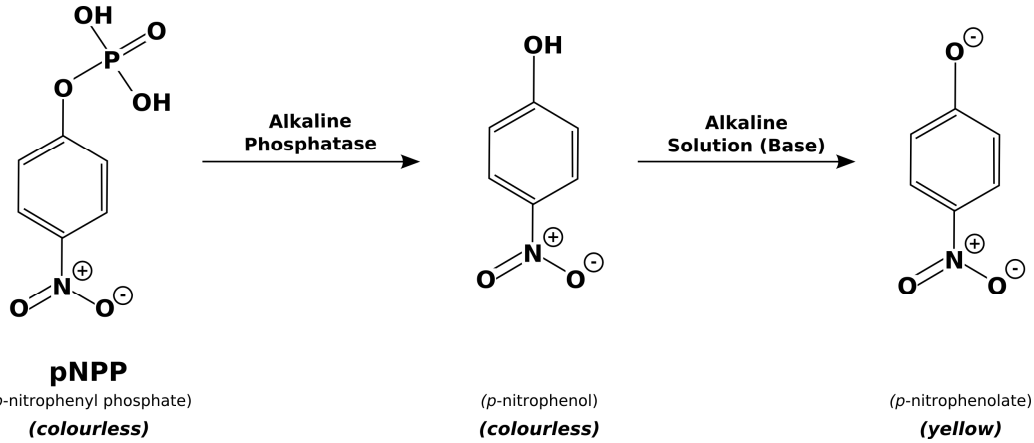


Figure 1: Secreted alkaline phosphatase catalyses the hydrolysis of pNitrophenyl phosphate (pNPP) producing a yellow product under alkaline conditions and can be conveniently measured at 405 nm on a spectrophotometer or an ELISA reader.

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.

This SEAP – Secreted Alkaline Phosphatase assay Kit provides a wide dynamic range, rapid results and is Fast, Easy, Accurate, Economical and Stable under storage conditions.

[1] Tate, S.S., Faseb Journal, 1990.

1.2. Kit Contents and Storage

Stability and Storage

Storage Upon receipt and for long-term use, store all reagent tubes at the indicated storage conditions (see table above). Kit's components are stable for at least 1 year at the recommended storage temperature.

Shipping condition: The SEAP – Secreted Alkaline Phosphatase assay Kit is shipped at RT.

The kit contains sufficient reagents to perform 500 assays in 96-well plate format.

Components	Quantity	Storage
pNPP-containing Assay Buffer (component A).	10 x 1 mL	-20°C
Reaction Buffer #1 (component B)	125 mL	4°C
Reaction Buffer #2 (component C)	125 µL	4°C
Dilution Buffer (component D)	50 mL	4°C

2. Applications and Protocols

2.1. 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 CO_2 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

2.2. 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 µL
25 wells	0.5 mL	4.5 mL	5 µL
50 wells	1.0 mL	9.0 mL	10 µL
75 wells	1.5 mL	13.5 mL	15 µL
100 wells (96 well-plate)	2.0 mL	18 mL	20 µL
1 well[1]	0.02 mL	0.18 mL	0.2 µL

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

2.3. 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 µL of sample to the corresponding wells of a 96-well plate.
6. Add 10 µL per well of Dilution Buffer (component D).

Performing the assay

1. Add 200 µL 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.

2.4. 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

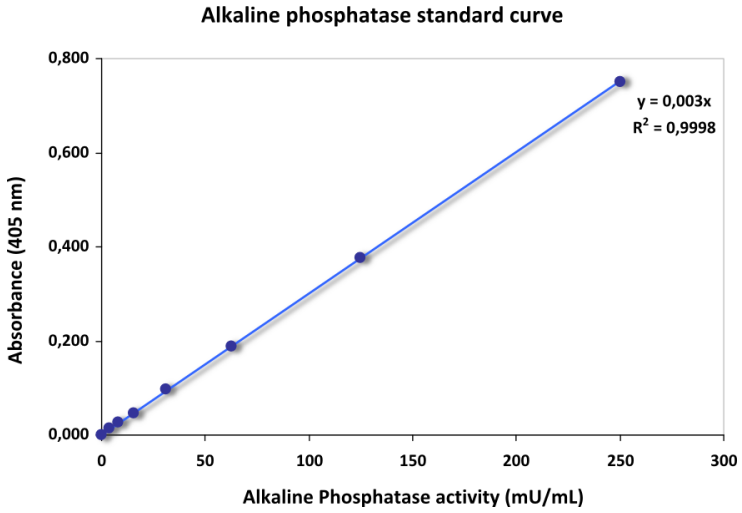


Figure 2: Alkaline phosphatase standard curve. A serial two-fold dilution of alkaline phosphatase standard was prepared and the absorbance was measured at 405 nm.

3. Related Products

Description
MAGNETOFECTION TECHNOLOGY
Super Magnetic Plate *(standard size for all cell culture support)* Mega Magnetic plate *(mega size to hold 4 culture dishes at one time)*
Transfection reagents:
PolyMag Neo (*for all nucleic acids*)
Magnetofectamine™ kit: Lipofectamine™ 2000 + CombiMag (*for all nucleic acids*)
NeuroMag *(dedicated for neurons)*
SilenceMag *(for siRNA application)*
Transfection enhancer:
CombiMag *(to improve any transfection reagent efficiency)*
Viral Transduction enhancers:
ViroMag (*to optimize viral transduction*)
ViroMag R/L *(specific for Retrovirus and Lentivirus)*
AdenoMag *(for Adenoviruses)*
In vivo Magnetofection
In vivo ViroMag (for magnetic assisted viral infection)
In vivo PolyMag (polymer-based magnetic nanoparticles)
In vivo DogtorMag (lipid-based magnetic nanoparticles)
LIPOFECTION TECHNOLOGY (LIPID-BASED)
Lullaby (*siRNA transfection reagent)*
DreamFect Gold *(Transfection reagent for all types of nucleic acids)*
VeroFect *(for Vero cells)*
Ecotransfect *(Economical reagent for routine transfection)*
FlyFectin *(for Insect cells)*
i-MICST TECHNOLOGY
Viro-MICST (*to transduce directly on magnetic cell purification columns)*
3D TRANSFECTION TECHNOLOGY
3DfectIN *(for hydrogels culture)*
3Dfect *(for scaffolds culture)*
RECOMBINANT PROTEIN PRODUCTION
HYPE-5 Transfection Kit (*for **H**igh **Y**ield **P**rotein **E**xpression)*
PROTEIN DELIVERY SYSTEMS
Ab-DeliverIN *(delivery reagent for antibodies)* Pro-DeliverIN *(delivery reagent for protein in vivo and in vitro)*
PLASMIDS PVECTOZ
pVectOZ-LacZ / pVectOZ-SEAP / pVectOZ-GFP / pVectOZ-Luciferase
ASSAY KITS
Bradford – Protein Assay Kit MTT cell proliferation kit β-Galactosidase assay kits (CPRG/ONPG)
BIOCHEMICALS
D-Luciferin, K^+ and Na^+ 1g G-418, Sulfate 1g
X-Gal powder 1g

Do not hesitate to contact us for all complementary information and remember to visit our website in order to stay inform on our last breakthrough technologies and updated on our complete product list. http://www.ozbiosciences.com

Purchaser Notification

Limited License

The purchase price paid for the SEAP – Secreted Alkaline Phosphatase assay Kit by end users grants them a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in the section 1, Kit Contents). This reagent is intended **for internal research only** by the buyer. Such use is limited to the use in the product manual. Furthermore, 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.

Purchasers may terminate this License at any time by returning all SEAP – Secreted Alkaline Phosphatase assay Kit material and documentation to OZ Biosciences, or by destroying all SEAP – Secreted Alkaline Phosphatase assay Kit 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 obtain a refund and/or to expressly terminate a research only license granted through the purchase of the kit(s).

This document covers in full 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

The 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 handling of the kit components by following appropriate research lab practices.

For more information, or for any comments on the terms and conditions of this License, please contact:

Director of Business Development
OZ Biosciences SAS
Parc Scientifique et Technologique de Luminy
Bâtiment Grand Luminy technopole
Case 922 zone entreprises
13288 Marseille Cedex 9, France
Ph: +33 (0)4.86.94.85.16
Fax: +33 (0)4.86.94.85.15
E-mail: contact@ozbiosciences.com

CONTACTS

OZ Biosciences SAS
163 avenue de Luminy
Case 922, zone entreprise
13288 Marseille cedex 09
FRANCE

Ph: +33 (0) 486 948 516
Fax: +33 (0) 486 948 515

contact@ozbiosciences.com
order@ozbiosciences.com
tech@ozbiosciences.com

OZ Biosciences INC
4901 Morena Blvd,
Suite 501
San Diego CA 92117
USA

Ph : + 1-858-246-7840
Fax : + 1-855-631-0626

contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com
techUSA@ozbiosciences.com

www.ozbiosciences.com

Follow us!