

Recombinant SEAP Protein

Recombinant human secreted embryonic alkaline phosphatase

Catalog # rec-hseap

For research use only, not for diagnostic or therapeutic use

Version #12A03-MM

PRODUCT INFORMATION

Content

10 μ g lyophilized recombinant human secreted embryonic alkaline phosphatase (SEAP)

Formulation

91 mM glycine, 91 mM TRIS and 5% w/v saccharose

Purity

> 90% determined by SDS-PAGE Coomassie Blue under denaturing conditions

Specific activity

> 100 units/ μ g

Unit definition: One unit is defined as the amount of enzyme that will hydrolyze 1 μ mole of 5-Bromo-4-chloro-3-indolyl phosphate (BCIP) per minute per μ g of protein at 37°C.

Product resuspension

Add 500 μ l of water to obtain a concentration of 20 μ g/ml

Storage

- Product is shipped at room temperature. Lyophilized product should be stored at -20°C.

- Upon resuspension, prepare aliquots of product and store at 4°C for 1 month or -20°C for 3 months. Avoid repeated freeze-thaw cycles.

DESCRIPTION

Recombinant SEAP protein is a truncated form of human placental alkaline phosphatase that comprises 520 amino acids. It is expressed in CHO cells and shows a 75 kDa band on SDS page. Recombinant SEAP protein is purified by affinity chromatography. Recombinant SEAP protein is a positive control for SEAP reporter assays.

SEAP is a reporter widely used to study promoter activity or gene expression. SEAP is secreted into cell culture supernatant and therefore offers many advantages over intracellular reporters. It allows to determine reporter activity without disturbing the cells, does not require the preparation of cell lysates and can be used for kinetic studies.

APPLICATIONS

Recombinant SEAP protein can be used as an internal positive control and as a reference to produce accurate standard curves with:

- **QUANTI-Blue™**, colorimetric enzyme assay developed to determine any alkaline phosphatase activity in a biological sample, such as supernatants of cell cultures,
- **SEAP Reporter Assay Kit**, a method to determine the levels of SEAP produced by cells transfected with a pNiFty SEAP reporter, or with a SEAP-expression plasmid, such as pSELECT-zeo-SEAP.

METHOD

Below is a protocol for using recombinant SEAP protein with QUANTI-Blue™. The following protocol refers to the use of 96-well plates. Vary your procedure accordingly depending on volumes of reagents needed based on the size of your wells. Dilute recombinant SEAP protein in culture medium containing heat-inactivated fetal bovine serum (FBS). Prepare QUANTI-Blue™ according to the instructions on the pouch.

- Add 180 μ l QUANTI-Blue™ per well.
- Add 20 μ l recombinant SEAP protein at different concentrations (suggested dose-response range 0.1 ng - 1 μ g/ml) or cell culture medium as a negative control.

Note: If the negative control turns purple/blue, it means your FBS contains alkaline phosphatase. We recommend to heat the FBS used in your cell culture medium at 56°C for 30 minutes to inactivate the alkaline phosphatase activity.

- Incubate at 37°C.
- After 15 min to 6 h incubation, assess SEAP activity with the naked eye or by reading the OD at 620-655 nm with a microplate reader.

RELATED PRODUCTS

Product	Catalog Code
QUANTI-Blue™	rep-qb1
SEAP Reporter Assay Kit	rep-sap
pSELECT-zeo-SEAP	psetz-seap
pNiFty-SEAP	pnifty-seap
RAW-Blue™ cells	raw-sp
THP1-XBlue™ cells	thpx-sp
HEK-Blue™ TLR cells	see website
HEK-Blue™ NOD cells	see website

For more information about InvivoGen's products see <http://www.invivogen.com>

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