pFUSE-SEAP-mG2bFc (mouse)

Control plasmid expression a mouse SEAP-Fc fusion protein

Catalog # pfuse-mg2bsp

For research use only

Version 20K04-MM

PRODUCT INFORMATION

Content:

- 20 µg of **pFUSE-SEAP-mG2bFc** plasmid provided as lyophilized DNA
- 1 ml of Zeocin™ (100 mg/ml)

Storage and Stability:

- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20˚C and is stable 3 months.
- Resuspended DNA should be stored at -20˚C and is stable up to 1 year.
- Store Zeocin™ at 4 °C or at -20 °C. The expiry date is specified on the product label.

Quality control:

- Plasmid construct has been confirmed by restriction analysis and sequencing.
- Plasmid DNA was purified by ion exchange chromatography and lyophilized.
- Expression of SEAP-mG2bFc was confirmed by using QUANTI-Blue™ Solution.
- SEAP-mG2bFc protein was purified using protein G affinity chromatography following manufacturer's protocol.

GENERAL PRODUCT USE

pFUSE-SEAP-Fc plasmids express a SEAP-Fc fusion protein generated by fusing the gene encoding for human secreted alkaline phosphatase (SEAP) and the Fc region of an immunoglobulin G (IgG).

pFUSE-SEAP-Fc plasmids yield high levels of Fc-Fusion proteins. The level of expression is usually in the µg/mL range. They can be transfected in a variety of mammalian cells, including myeloma cell lines, Chinese hamster ovary (CHO) cells, monkey COS cells and human embryonic kidney (HEK)293 cells. These cells are commonly used in protein purification systems.

SEAP-Fc fusion proteins are secreted and can be easily detected in the supernatant of pFUSE-SEAP-Fc-transfected cells by using QUANTI-Blue™ Solution, a SEAP detection medium.

SEAP-Fc fusion proteins can be easily purified by single-step protein A or protein G affinity chromatography.

PLASMID FEATURES

• **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter[1] and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat[2]. The EF-1α promoter exhibits a strong activity and yields long lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1α core promoter to enhance stability of RNA.

• **SEAP-mG2bFc** was generated by fusing the gene encoding for human SEAP with the Fc region of mouse IgG2b. This region comprises the CH2 and CH3 domains of the IgG heavy chain and the hinge region. The hinge serves as a flexible spacer between the SEAP and Fc moieties, allowing each part of the molecule to function independently.

• **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA[3].

• **ori:** a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.

• **CMV enh / hFerL prom:** This composite promoter combines the human cytomegalovirus immediate-early gene 1 enhancer and the core promoter of the human ferritin light chain gene. This ubiquitous promoter drives the expression of the Zeocin™-resistance gene in mammalian cells.

• **EM2KC** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*. EM2KC is located within an intron and is spliced out in mammalian cells.

• **Zeo:** Resistance to Zeocin™ is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus* The same resistance gene confers selection in both mammalian cells and *E. coli*.

• **ßGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription[4].

References:

1. Kim DW *et al.* 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. 91(2):217-23.
2. Takebe Y. *et al.* 1988. SR alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. Mol Cell Biol. 8(1):466-72.
3. Carswell S. & Alwine JC. 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol Cell Biol. 9(10):4248-58.
4. Yu J. & Russell JE. 2001. Structural and functional analysis of an mRNP complex that mediates the high stability of human beta-globin mRNA. Mol Cell Biol. 21(17):5879-88.

TECHNICAL SUPPORT
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METHODS

Plasmid resuspension
Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile H_2O. Store resuspended plasmid at -20 °C.

Plasmid amplification and cloning
Plasmid amplification and cloning can be performed in *E. coli* GT116 or in other commonly used laboratory *E. coli* strains, such as DH5α.

Zeocin™ usage
This antibiotic can be used for **E. coli** at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

Purification of SEAP-mG2bFc protein

The following protocol describes the purification of SEAP-mG2bFc protein produced by 293 cells using Protein G affinity chromatography.

1- Seed $3.5x10^6$ 293 cells in a 100 mm plate containing 6 ml of DMEM supplemented with 10% FBS.
2- Transfect cells with 750 µl of pFUSE-SEAP-mG2bFc/LyoVec™ complexes at a ratio of 1:6 prepared by mixing 7.5 µg pFUSE-SEAP-mG2bFc and 750 µl reconstituted LyoVec™ following the LyoVec™ protocol.
3- After 16 hours transfection, replace the medium with a serum-free medium such as PRO 293a-CDM (Biowithaker-Cambrex).
4- After 72 hours transfection, collect supernatant.
5- Purifiy protein using Protein G affinity chromatography such as Hi Trap Protein G HP (Amersham Biosciences) following manufacturer's protocol.

RELATED PRODUCTS

Product	Catalog Code
LyoVec™	lyec-1
QUANTI-Blue™ Solution	rep-qbs

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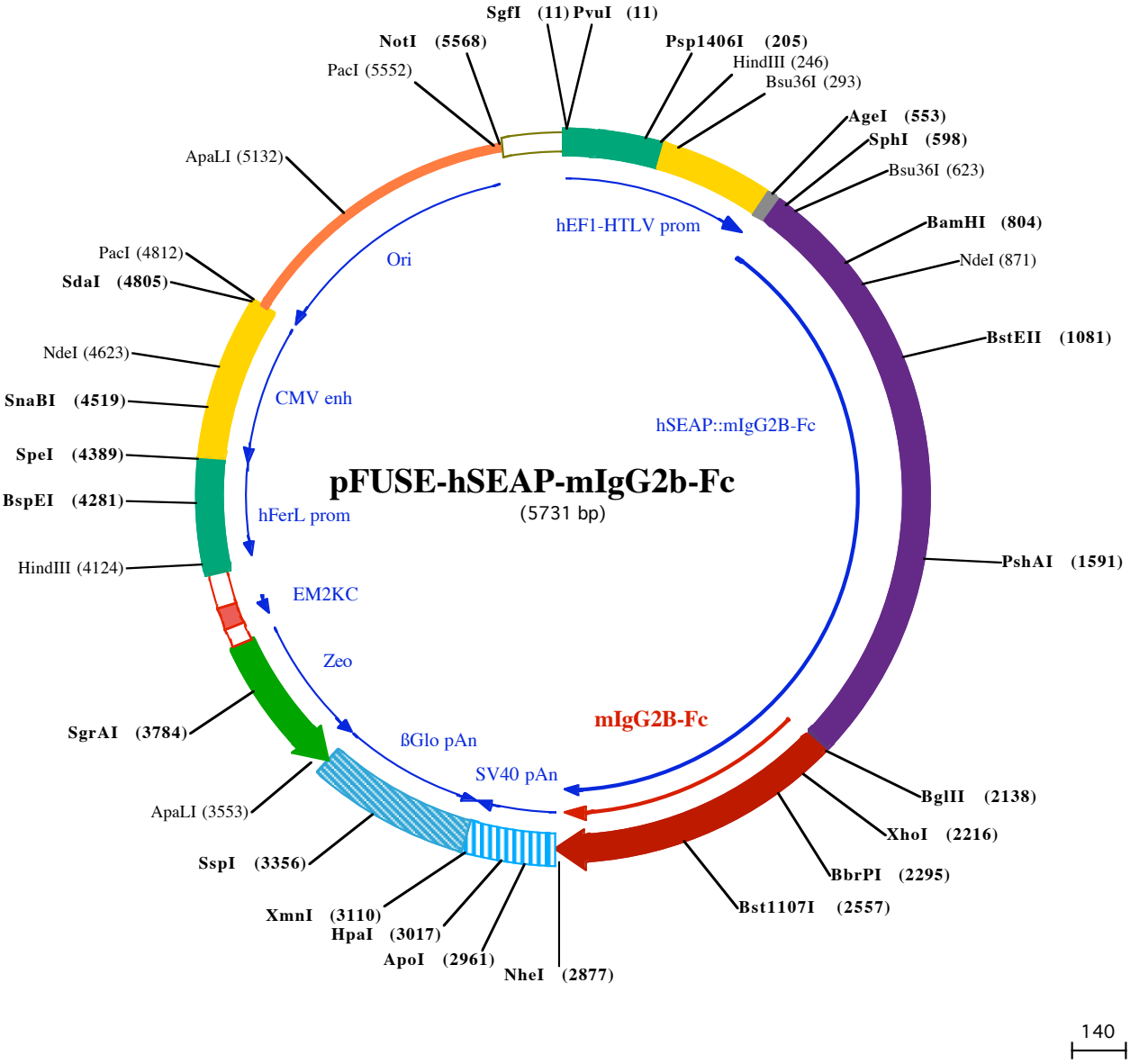
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SgfI (11)
PvuI (11)
NotI (5568)
PacI (5552)
Psp1406I (205)
HindIII (246)
Bsu36I (293)
AgeI (553)
SphI (598)
Bsu36I (623)
BamHI (804)
NdeI (871)
BstEII (1081)
PshAI (1591)
BglII (2138)
XhoI (2216)
BbrPI (2295)
Bst1107I (2557)
NheI (2877)
ApoI (2961)
HpaI (3017)
XmnI (3110)
SspI (3356)
ApaLI (3553)
SgrAI (3784)
HindIII (4124)
BspEI (4281)
SpeI (4389)
SnaBI (4519)
NdeI (4623)
SdaI (4805)
PacI (4812)
ApaLI (5132)
hEF1-HTLV prom
Ori
CMV enh
hFerL prom
EM2KC
Zeo
ßGlo pAn
SV40 pAn
hSEAP::mIgG2B-Fc
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