pNiFty2-56K-SEAP

Inducible reporter plasmid selectable with Zeocin™

Catalog code: pnf2-56ksp

For research use only

Version 20L03-MM

PRODUCT INFORMATION

Contents:

• 20 µg of pNiFty2-56K-SEAP 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.
• Resuspended DNA should be stored at -20 °C and is stable for 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 full-length ORF sequencing.
• Plasmid DNA was purified by ion exchange chromatography.

GENERAL PRODUCT USE

Interferons are key modulators of the immune response. Their pleiotropic activities are mediated by the induction of many IFN-stimulated genes (ISGs). To help study the transcriptional regulation and signal transduction of type I IFNs, InvivoGen provides several reporter systems, called pNiFty2, based on the inducible expression of the secreted embryonic alkaline phophatase (SEAP) gene. The SEAP gene is cloned under the control of three different promoters that are activated by various transcription factors, such as IRF3, IRF5, IRF7 and NF-κB. pNiFty2-56K-SEAP features the human ISG-56K promoter.

PLASMID FEATURES

• **hISG-56K prom:** ISG-56K is the prototype ISG which expression is highly increased by Type I IFNs due to the presence of ISRE and IRF7 sites within the promoter[1]. Co-expression of IGSG-56K-SEAP with constitutively activated IRF3 (saIRF3) or IRF7 (saIRF7) in HEK293 cells led to a strong increase in SEAP expression.
• **5U-140** is a synthetic 5'UTR containing an intron.
• **SEAP** is a secreted form of human embryonic alkaline phosphatase. Unlike endogenous alkaline phosphatases, SEAP is extremely heat stable and resistant to the inhibitor L-homoarginine. It catalyses the hydrolysis of pNitrophenyl phosphate (pNpp) producing a yellow end product. SEAP expression can be readily quantified by collecting samples of culture medium and measuring the hydrolysis of pNpp with a spectrophotometer at 405 nm.
• **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA.
• **hEF1/HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter[2] 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[3]. 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.
• **EM7** is a bacterial promoter that enables the constitutive expression of the Zeocin™ antibiotic resistance gene in *E. coli*.
• **Zeo:** Resistance to the antibiotic Zeocin™ is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus*. The *Sh ble* gene is driven by the CMV enhancer/promoter in tandem with the bacterial EM7 promoter allowing 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].
• **pMB1 Ori** is a minimal *E. coli* origin of replication with the same activity as the longer Ori.

1. Der SD. *et al.* 1998. Identification of genes differentially regulated by interferon alpha, beta, or gamma using oligonucleotide arrays. PNAS. 95(26):15623-8. **2. Kim DW. *et al.*, 1990.** Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. Gene 91(2): 217-23. **3. Takebe Y. *et al.*, 1988.** SR alphapromoter: an efficient and versatile mammalian cDNA expression system composed ofthe simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virustype 1 long terminal repeat. Mol Cell Biol. 8(1):466-72. **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.

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.

TECHNICAL SUPPORT
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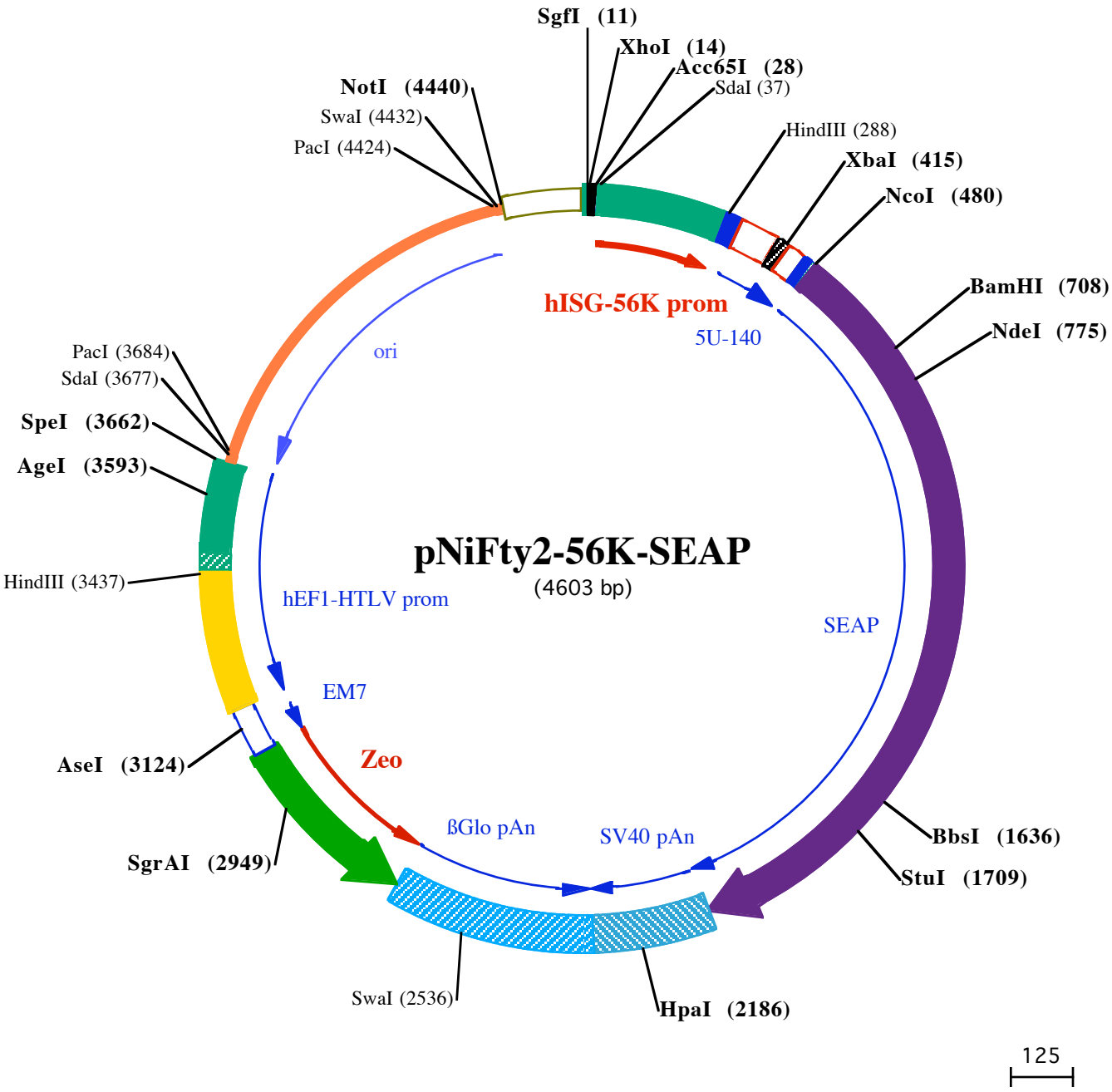
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Acc65I (28)
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NotI (4440)
SwaI (4432)
PacI (4424)
HindIII (288)
XbaI (415)
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hISG-56K prom
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pNiFty2-56K-SEAP
(4603 bp)
hEF1-HTLV prom
SEAP
EM7
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AseI (3124)
ßGlo pAn
SV40 pAn
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BbsI (1636)
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