pUNO1-<Gene>-HA3x

Expression vector containing a HA-tagged fully sequenced open reading frame

Catalog code: puno1ha-<gene>

https://www.invivogen.com/genes

For research use only

Version 19K10-MM

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized plasmid DNA
- 2 x 1 ml blasticidin at 10 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 at least for 1 year.
- Store blasticidin at 4°C or -20°C. *

*The expiry date is specified on the product label.

Quality control

- Plasmid construct has been confirmed by restriction analysis and full-length open reading frame (ORF) sequencing.
- Plasmid DNA was purified by ion exchange chromatography.

GENERAL PRODUCT USE

• **Obtaining a gene to subclone into another vector.** The gene of interest is flanked by two unique restriction sites allowing its convenient excision. These restriction sites are compatible with other restriction sites contained in multiple cloning sites, thus facilitating subcloning.

• **Stable gene expression in mammalian cells.** pUNO1 plasmids can be used directly in transfection experiments both in vitro and in vivo. pUNO1 plasmids contain the blasticidin-resistance gene (bsr) driven by the CMV promoter/enhancer in tandem with the bacterial EM7 promoter. This allows the amplification of the plasmid in E. coli, as well as the selection of stable clones in mammalian cells using the same selective antibiotic. pUNO1 allows high levels of expression and secretion (where applicable) of the gene product.

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 water. Store resuspended plasmid at -20°C.

Plasmid amplification and cloning

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

Blasticidin usage

Blasticidin should be used at 25-100 µg/ml in bacteria and 1-30 µg/ml in mammalian cells. Blasticidin is supplied at 10 mg/ml in HEPES buffer.

Preparation of cell extract for detection of HA-tagged proteins:

The buffer used to prepare the cell lysates is a modified RIPAbuffer that is suitable for recovery of membrane receptors.

1. Lift cells from 6-well plate and wash twice with PBS.
2. Add 100µl of Cell Lysis Buffer and incubate 30 min. on ice.
3. Centrifuge 10 min at 10,000 rpm (at 4˚C if possible).
4. Transfer supernatant to a new tube and store at -20˚C.

Cell Lysis Buffer: 5ml of 100mM Tris HCl pH 7.4, 5ml of 1M NaCl, 100µl of 0.5M EDTA, 500µl of 100mM NaF, 500µl of 10% SDS, 2.5ml of 10% Na-Deoxycholate, 500µl of 100% Triton-X100, 5ml of 10% Glycerol, H2O to a final volume of 50ml. Store this stock buffer at 4˚C. Determine amount of lysis buffer needed to make cell extracts and just before use add the following components at the final concentrations of 1mM PMSF, 1:100 Protease Inhibitor cocktail, 2mM of Na_3VO_4.

PLASMID FEATURES

• **EF-1α/HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) core promoter[1] and the 5' untranslated region of the Human T-Cell Leukemia Virus (HTLV). EF-1α utilizes a type 2 promoter that encodes for a «house keeping» gene. It is expressed at high levels in all cell cycles and lower levels during G0 phase. The promoter is also non-tissue specific; it is highly expressed in all cell types. The R segment and part of the U5 sequence (R-U5') of the HTLV Type 1 Long Terminal Repeat[2] has been coupled to the EF-1α promoter to enhance stability of DNA and RNA. This modification not only increases steady state transcription, but also significantly increases translation efficiency possibly through mRNA stabilization.

• ORF: pUNO1 provides an intronless ORF from the ATG to the stop codon, fully-sequenced, and typically flanked by convenient cloning sites for easy subcloning. Typically, the 5' end of the ORF contains a unique NcoI, BspHI, BspLU11I, or SphI site encompassing the ATG Start codon. When this 5' cloning site is not unique, another restriction (e.g. AgeI) is added a few bases upstream of the ATG. The 3' end of the ORF contains a unique NheI site (or compatible site) after the Stop codon.

- AgeI is compatible with XmaI, BspEI, NgoMIV and SgrAI.
- NcoI is compatible with BspHI and BspLU11I.
- NheI is compatible with XbaI, SpeI, and AvrII.

The ORF is fused to three **influenza hemagglutinin epitope tags** (YPYDVPDYA x3) at the 3'end, that allows for simple and convenient detection of the expressed gene by Western blot using anti-HA antibody.

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

• **pMB1 ori** is a minimal E. coli origin of replication to limit vector size, but with the same activity as the longer Ori.

• **CMV promoter & enhancer** drives the expression of the blasticidin resistance in mammalian cells.

• Bsr (blasticidin resistance gene): The bsr gene from Bacillus cereus encodes a deaminase that confers resistance to the antibiotic blasticidin. The bsr gene is driven by the CMV promoter/enhancer in tandem with the bacterial EM7 promoter. Therefore, blasticidin can be used to select stable mammalian cells transfectants and E. coli transformants.

• **Human beta-Globin polyA** is a strong polyadenylation (pAn) signal placed downstream of bsr. The use of beta-globin pAn minimizes interference[4] and possible recombination events with the SV40 polyadenylation signal.

1. Kim DW. *et al.*, 1990. Use of the human elongation factor 1α promoter as a versatile and efficient expression system. Gene 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 β-globin mRNA. Mol Cell Biol. 21(17):5879-88.

RELATED PRODUCTS

Product	Description	Cat. Code
Blasticidin	Selection antibiotic	ant-bl-1
ChemiComp GT116	Competent *E. coli*	gt116-11

TECHNICAL SUPPORT
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