

# pUNO2-<Gene>

Expression vector containing a fully sequenced open reading frame

Catalog code: puno2-<gene>

<https://www.invivogen.com/genes>

For research use only

Version 21E20-MM

## PRODUCT INFORMATION

### Contents

- 20 µg of lyophilized plasmid DNA
- 1 ml of Zeocin™ at 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 at least for 1 year.
  - Store Zeocin™ 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,** pUNO2 plasmids can be used directly in transfection experiments both in vitro and in vivo. pUNO2 plasmids contain the Zeocin™-resistance gene (*Sh ble*) 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. pUNO2 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.

### Zeocin™ usage

Zeocin™ should be used at 25 µg/ml in bacteria and 50-400 µg/ml in mammalian cells. Zeocin™ is supplied as 100 mg/ml solution in HEPES buffer.

## PLASMID FEATURES

- **EF-1α/HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) core promoter<sup>1</sup> 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<sup>2</sup> 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:** pUNO2 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, BspLU111, 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 BspLU111.
  - NheI is compatible with XbaI, SpeI, and AvrII.
- **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions, resulting in high levels of steady-state mRNA<sup>3</sup>.
- **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 Zeocin™ resistance in mammalian cells.
- **Sh ble (Zeocin™ resistance gene):** Resistance to Zeocin™ is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus*. The *Sh ble* gene is driven by the CMV promoter/enhancer in tandem with the bacterial EM7 promoter. Therefore, Zeocin™ 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 *Sh ble*. The use of beta-globin pAn minimizes interference<sup>4</sup> 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
Zeocin™	Selection antibiotic	ant-zn-1
ChemiComp GT116	Competent <i>E. coli</i>	gt116-11

## TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873  
InvivoGen USA (International): +1 (858) 457-5873  
InvivoGen Europe: +33 (0) 5-62-71-69-39  
InvivoGen Asia: +852 3622-3480  
E-mail: [info@invivogen.com](mailto:info@invivogen.com)

