pUNO1-<Gene>
Expression vector containing a fully sequenced open reading frame
Catalog code: puno1-<gene>
https://www.invivogen.com/genes
For research use only
Version 19K09-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.

Contents
- 2 x 1 ml blasticidin at 10 mg/ml

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 as a 10 mg/ml colorless solution in HEPES buffer.

PLASMID FEATURES
- **EF-1α/HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) core promoter1 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 US sequence (R-US) of the HTLV Type I Long Terminal Repeat (LTR)1 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 Nhel or compatible site after the Stop codon.
- **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions, resulting in high levels of steady-state mRNA2.
- **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:** a strong polyadenylation (pAn) signal placed downstream of bsr. The use of beta-globin pAn minimizes interference with the SV40 polyadenylation signal.


RELATED PRODUCTS

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<tr>
<th>Product</th>
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<tr>
<td>Blasticidin</td>
<td>Selection antibiotic</td>
<td>ant-bl-1</td>
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<tr>
<td>ChemiComp GT116</td>
<td>Competent E. coli</td>
<td>gt116-11</td>
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