**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**

- **Subclone gene into another vector.** Two unique restriction sites flank the gene, allowing convenient excision. The 5’ site is BspEI which is compatible with AgeI, Xmal, NcoMIV and SgrAI. The 3’ site is Nhel which is compatible with Xbal, SpeI, and AvrII.
- **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 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.

**PLASMID FEATURES**

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

**Human STING-MRP**

- **ORF size:** 852 bp
- **Cloning fragment size:** 961 bp

**STING** (stimulator of interferon genes; also known as TMEM173, MITA, MPPS, and ERIS) is essential for the IFN response to microbial or self-DNA, and acts as a direct sensor of cyclic dinucleotides (CDNs). CDNs are important messengers in bacteria, affecting numerous responses of the prokaryotic cell, but also in mammalian cells, acting as agonists of the innate immune response. hSTING-MRP (MITA-related protein), discovered and identified in HEK293T cells, is an alternatively spliced isoform of hSTING lacking exon 7 that acts as a dominant negative mutant of STING. It was recently reported to block STING-mediated IFN response while retaining the ability to activate NF-κB.

**EF-1α/HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α core promoter 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 1 Long Terminal Repeat 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.

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

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

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


**RELATED PRODUCTS**

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<td>Selection antibiotic</td>
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<td>ChemiComp GT116</td>
<td>Competent E. coli</td>
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