**pUNO1-<Gene>-HA3x**

Expression vector containing a fully sequenced open reading frame.

Catalog # puno1 ha-<gene>

For research use only

Version # 14A23-JC

**PRODUCT INFORMATION**

**Contents:**
- 20 µg of lyophilized plasmid DNA
- 4 pouches of *E. coli* Fast-Media® Blas (2 TB and 2 Agar)
- 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 up to 1 year.
- Store *E. coli* Fast-Media® at room temperature in a dry and cool place. Fast-Media® pouches are stable 2 years when stored properly.
- Store blasticidin at 4°C or -20°C for up to two years. Product is stable 2 weeks at 37°C. Avoid repeated freeze-thaw cycles.

**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**

- 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** can be performed in *E. coli* GT116 or other commonly used laboratory *E. coli* strains, such as DH5α.

**Selection of bacteria with *E. coli* Fast-Media® Blas:** *E. coli* Fast-Media® Blas is a fast and convenient way to prepare liquid and solid media for bacterial culture by using only a microscope. See detailed protocol overleaf.

**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.

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

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

1. Lift cells from 6-well plate and wash twice with PBS.
2. Add 100 µl 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:**
- 50mM Tris HCl pH 7.4, 5mM of 1M NaCl, 100µg of 0.5M EDTA, 500µl of 100mM NaF, 500µg of 10% SDS, 2.5ml of 10% Na-Deoxycholate, 500g/l 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 Na3VO4.

**PLASMID FEATURES**

**EF-1α / HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) core promoter and the 5’s 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 types down to 20°C 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 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 3' end of the ORF contains a unique NcoI, BspHII, BspLU111, or SpII site encompassing the ATG Start codon. When this 5' cloning site is not unique, another restriction site (e.g. AgeI) is added to add 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 Xmal, BspEI, NgoMIV and SgrAI.
- NcoI is compatible with BspHII and BspLU111.
- NheI is compatible with Xbal, 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 an HA primary antibody. InvivoGen offers an Anti-HATag (catalog code: ab-hatag) that can be used to detect the expressed fusion protein.

**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.

**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 with possible recombination events with the SV40 polyadenylation signal.


Blasticidin
Selective antibiotic for the bsr or BSD genes

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PRODUCT INFORMATION
Contents:
Blasticidin hydrochloride is supplied as 1 ml tubes of a 10 mg/ml colorless solution in HEPES buffer (100% active compound), pH 7.5, filtered to sterility for customer convenience and cell culture tested.
Quality control:
Purity controlled by HPLC: >95%. Activity controlled by bioassays on bacteria and mammalian cell lines.

SPECIAL HANDLING
Blasticidin is a hazardous compound. Avoid contact with eyes, skin and clothes.

BACKGROUND
Blasticidin is a peptidyl nucleoside antibiotic isolated from the culture broth of Streptomyces griseochromogenes. It specifically inhibits protein synthesis in both prokaryotes and eukaryotes through inhibition of peptide bond formation in the ribosomal machinery. Blasticidin is used to select transfected cells carrying bsr or BSD resistance genes.

CAS number: 3513-03-9
Formula: C17H26N8O5, HCl
Molecular weight: 458.9
pKa values: 2.8, 4.2, 8.2 and 12.5

RESISTANCE TO BLASTICIDIN
Three blasticidin resistance genes have been cloned and sequenced: an acetyl transferase gene, bls from a blasticidin producer strain, and two deaminase genes, bsr gene from Bacillus cereus, and BSD gene from Aspergillus terreus. Both bsr and BSD genes are used as dominant selectable markers for gene transfer experiments in mammalian and plant cells. Although blasticidin was developed as a selection agent for mammalian cells, the bsr and BSD resistance genes can also be used in E. coli.

CONDITIONS OF SELECTION
- Escherichia coli is poorly sensitive to blasticidin, but transformants resistant to blasticidin can be selected on low salt LB agar medium, pH 8, supplemented with 100 µg/ml blasticidin. High pH enhances activity of blasticidin. For optimum results, the use of InvivoGen's Fast-Media® Blas is recommended.
- Mammalian cells: The working concentration of blasticidin for mammalian cell lines varies from 1 to 10 µg/ml (ex. HeLa, HEK293, B16), in a few cases up to 30 µg/ml (ex. PC1.0). In a starting experiment we recommend to determine optimal concentrations of antibiotic required to kill your host cell line. After treatment, cell death occurs rapidly, as fast as G418 selection, allowing the selection of transfected cells with plasmids carrying the bsr or BSD genes in as little as 7 days post-transfection.

Note: Antibiotics work best when cells are actively dividing. If the cells become too dense, the antibiotic efficiency will decrease. It is best to split cells such that they are no more than 25% confluent.

FAST-MEDIA® Features
Fast-Media® offer researchers a quick and convenient way to prepare 200 ml of sterile E. coli growth medium in about five minutes using a microwave instead of an autoclave.

Fast-Media® is available with a large choice of antibiotics for selection, and chromogenic substrates, for the growth or selection of E. coli transformant colonies, as well as detection of blue/white colonies. Fast-Media® Base is supplied without selectable antibiotics. See the variety of available Fast-Media® products at http://www.invivogen.com/fast-media.