pUNO1-<Gene>-HA3x

Expression vector containing a fully sequenced open reading frame.

Catalog # puno1ha-<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 microwave. 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 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 Na3VO4 .

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 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[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.

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Blasticidin

Selective antibiotic for the *bsr* or *BSD* genes

For research use only

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 bound formation in the ribosomal machinery. Blasticidin is used to select transfected cells carrying *bsr* or *BSD* resistance genes.

CAS number: 3513-03-9

Formula: $C_{17}H_{26}N_8O_5$, 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[1], and two deaminase genes, *bsr* gene from *Bacillus cereus*[2,3], and *BSD* gene from *Aspergillus terreus*[4,5]. 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® Blas

Microwaveable media for selection and propagation of blasticidin-resistant *E. coli*

For research use only

PRODUCT INFORMATION

Contents:

Each Fast-Media® Blas pouch contains the necessary amount of powder for the preparation of **200 ml** of medium supplemented with **blasticidin**. Agar media is LB-based (Lysogeny Broth also known as Luria Broth), liquid media is TB-based (Terrific Broth).

Effective concentration: Blasticidin 100 µg/ml

METHOD

For customer convenience, the following procedure is directly printed on each pouch.

1. Pour the pouch contents into a clean borosilicate glass bottle or flask.
2. Add 200 ml of distilled or deionized water.
3. Mix thoroughly by swirling the glass bottle or flask.
4. Heat in a microwave oven on MEDIUM power setting (about 450W) until bubbles start to appear (about 3 minutes).

Do not heat in a closed container.

5. Swirl gently to mix the preparation and re-heat for 30 seconds. Swirl gently again.
6. Repeat step 4 if necessary until the medium is completely dissolved. Do not overboil.
7. Allow the medium to cool to 50-55°C before use.

Caution: *Any solution heated in a microwave oven may become superheated and suddenly boil when moved or touched. Handle with extreme care. Wear heat-proof gloves.*

Note: *Do not repeat this above procedure once the medium is prepared because the antibiotic will be adversely affected.*

SPECIAL HANDLING

Caution should be exercised during handling of Fast-Media® due to potential allergenic properties of antibiotics. Wear protective gloves, do not breathe the dust.

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 selective antibiotics.

See the variety of available Fast-Media® products at http://www.invivogen.com/fast-media.

1. Perez-Gonzalez JA. et *al.*, 1990. Cloning and characterization of the gene encoding a blasticidin S acetyltransferase from *Streptoverticillum sp*. Gene 86:129-34. **2. Izumi M. et *al.*, 1991.** Blasticidin S-resistance gene (*bsr*): A novel selectable marker for mammalian cells. Exp. Cell Res. 197:229-33. **3. Itaya M. et *al.*, 1990.** The blasticidin S resistance gene (*bsr*) selectable in a single copy state in the *Bacillus subtilis* chromosome. J. Biochem. 107: 799-801. **4. Kimura M. et *al.*, 1994.** Cloning of the blasticidin S deaminase gene (BSD) from Aspergillus terreus and its use as a selectable marker for *Schizosaccharomyces pombe* and *Pyricularia oryzae*. Mol. Gen. Genet. 242:121-9. **5. Kimura M. et *al.*, 1994.** Blasticidin S deaminase gene from *Aspergillus terreus* (BSD): a new drug resistance gene for transfection of mammalian cells.Biochim. Biophys. Acta. 1219:653-9.

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