# pUNO2-<Gene>

Expression vector containing a fully sequenced open reading frame

Catalog # puno2-<gene>

For research use only

Version # 15H19-MM

## PRODUCT INFORMATION

**Contents:**

- 20 µg of lyophilized plasmid DNA
- 4 pouches of *E. coli* Fast-Media® Zeo (2 TB and 2 Agar)
- 1 ml Zeocin™ at 100 mg/ml

**Storage and Stability:**

- Product is shipped at room temperature.
- Store lyophilized DNA at -20 °C.
- Resuspended DNA is stable up to 1 year when stored at -20 °C.
- Store *E. coli* Fast-Media® at room temperature in a dry and cool place. Fast-Media® pouches are stable for 2 years when stored properly.
- Store Zeocin™ at 4 °C or -20 °C for up to 18 months. 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. 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.

**Selection of bacteria with *E. coli* Fast-Media® Zeo:**

*E. coli* Fast-Media® Zeo is a fast and convenient way to prepare liquid and solid media for bacterial culture by using only a microwave. See detailed protocol overleaf.

**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[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:** 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, 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.

**• 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 hygromycin resistance in mammalian cells.

**• EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli.*

**• Sh ble (Zeocin™ resistance gene):** Resistance to Zeocin™ is conferred by the S*h ble* gene from *Streptoalloteichus hindustanus*. The S*h 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 S*h ble*. 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.

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 Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com

# Zeocin™

Selective antibiotic for the *Sh ble* gene

For research use only

## PRODUCT INFORMATION

**Contents:**
Zeocin™ is supplied as either 1 ml tubes or a 50 ml bottle of a 100 mg/ml solution (100% active product) in HEPES buffer, pH 7.25, filtered to sterility for customer convenience, and validated for cell-culture usage.
**CAS number:** 11006-33-0
**Quality control:**
Purity: >90% (HPLC)
Activity is tested using microbiological assays

## BACKGROUND

Zeocin™ is the commercial name of a special formulation containing Phleomycin D1, a copper-chelated glycopeptide antibiotic isolated from culture broth of a *Streptomyces verticillus* mutant. This antibiotic exhibits activity against bacteria, eukaryotic microorganisms, plant and animal cells. Due to its broad spectrum of toxicity, Zeocin™ is useful for the selection of cell carrying Zeocin™ resistance genes.

## RESISTANCE TO HYGROMYCIN

The Zeocin™ resistance gene (*Sh ble* gene) encodes a small protein (14 kDa) whose structure has been characterized[1, 2]. The *Sh ble* protein appears to be non-toxic for a wide variety of cells in which the gene was expressed. This protein binds Zeocin™ with a strong affinity. The binding of Zeocin™ inhibits its DNA strand cleavage activity. As there is no cross resistance with other currently used drug resistance markers, Zeocin™ can be used to select cells resistant to other selective agents (i.e. G418, hygromycin B, blasticidin S or puromycin).

**1. Gatignol A. et al., 1988.** Bleomycin resistance conferred by a drug-binding protein. FEBS Letters. 230: 171-5. **2. Dumas P. et al., 1994.** The three dimensional structure of a bleomycin resistance protein. Embo J. 242 (5) 595-601.

## CONDITIONS OF SELECTION

**- *Escherichia coli*** The Sh ble gene and the hybrid genes in vectors provided by InvivoGen are driven by synthetic *E. coli* promoters (i.e. EM7). The cells of the common *E. coli* recipient strains (i.e. HB101, DH5α, MC1061) transformed by these vectors are resistant to Zeocin™.
*Note: Do not use an E. coli recipient strain that contains the Tn5 transposable element (i.e. MC1066). Tn5 encodes a bleomycin-resistance gene that will confer resistance to Zeocin™.*
Zeocin-resistant transformants are selected in Low Salt LB agar medium (yeast extract 5 g/l, Tryptone 10 g/l, NaCl 5 g/l, Agar 15 g/l, pH 7.5) supplemented with 25 µg/ml of Zeocin™. Plates containing Zeocin™ are stable for 1 month when stored at 4 °C. For optimum results the use of InvivoGen's FastMedia® Zeo is recommended.
**- Mammalian cells**: The working concentration of Zeocin™ for mammalian cell lines varies from 50 to 400 µg/ml, in a few cases can be as low as 20 µg/ml or as high as 1000 µg/ml. In a starting experiment we recommend to determine the optimal concentration of Zeocin™ required to kill your host cell line. The killing and the detachment of dead cells from the plate, especially at high cell density, can require a longer time compared to G418. Foci of Zeocin-resistant stable transfectants are usually individualized after 5 days to 3 weeks incubation, depending on the cell line.

# Fast-Media® Zeo

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

For research use only

## PRODUCT INFORMATION

**Contents:**
Each Fast-Media® Zeo pouch contains the necessary amount of powder for the preparation of **200 ml** of medium supplemented with **Zeocin™**. Agar media is LB-based (Lysogeny Broth also known as Luria Broth), liquid media is TB-based (Terrific Broth).
**Effective concentration:** Zeocin™ 25 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 450 W) 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.*

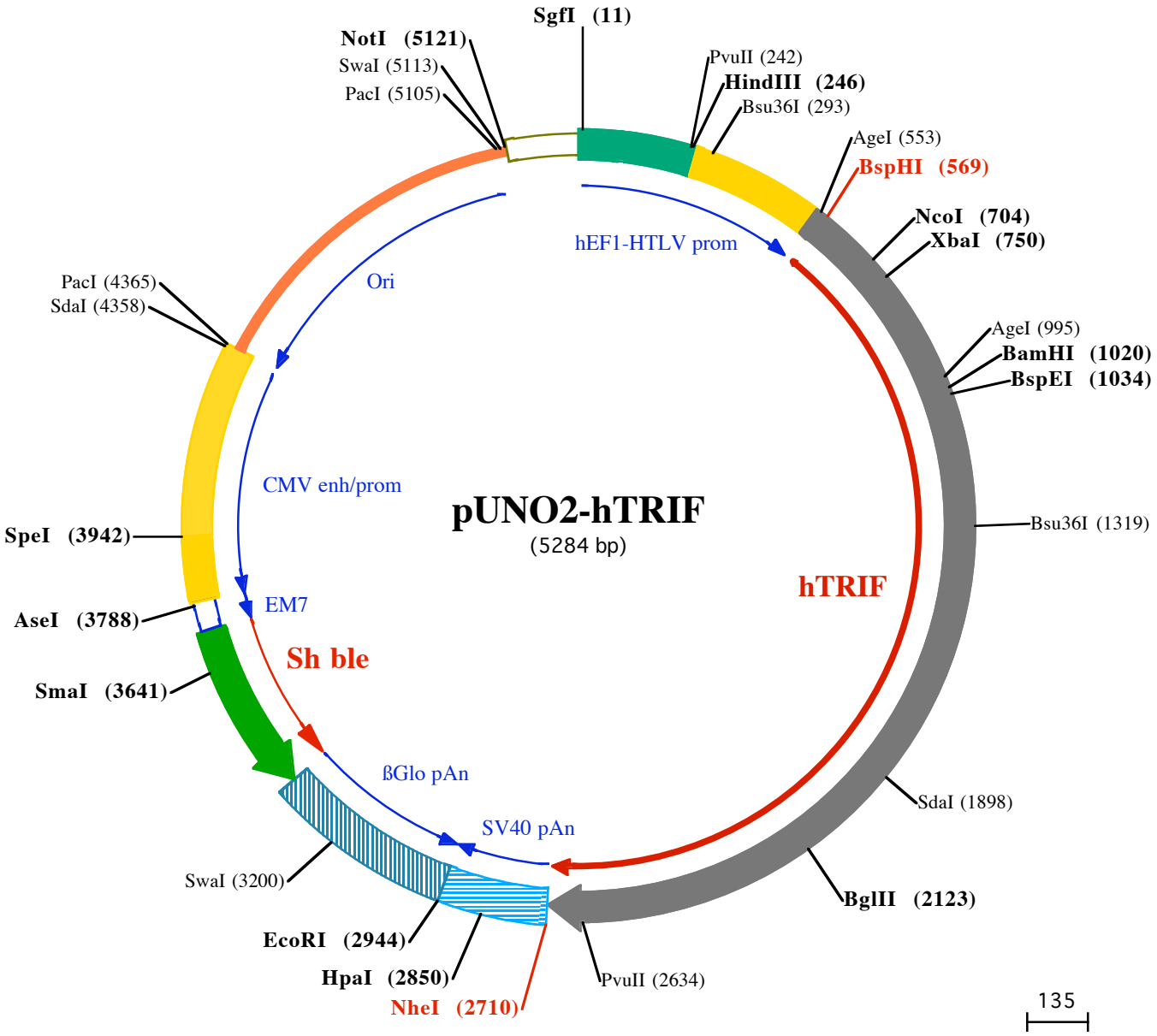
## SPECIAL HANDLING

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.

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 Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com


SgfI (11)
NotI (5121)
SwaI (5113)
PacI (5105)
PvuII (242)
HindIII (246)
Bsu36I (293)
AgeI (553)
BspHI (569)
NcoI (704)
XbaI (750)
hEF1-HTLV prom
Ori
PacI (4365)
SdaI (4358)
AgeI (995)
BamHI (1020)
BspEI (1034)
CMV enh/prom
pUNO2-hTRIF
(5284 bp)
Bsu36I (1319)
SpeI (3942)
hTRIF
EM7
AseI (3788)
Sh ble
SmaI (3641)
ßGlo pAn
SdaI (1898)
SV40 pAn
SwaI (3200)
BglII (2123)
EcoRI (2944)
HpaI (2850)
PvuII (2634)
NheI (2710)
135


SgfI (11)
HindIII (246)
PvuII (242)
Bsu36I (293)
AgeI (553)
BspHI (569)
NcoI (704)
XbaI (750)
AgeI (995)
BamHI (1020)
BspEI (1034)
Bsu36I (1319)
SdaI (1898)
BglII (2123)
PvuII (2634)
NheI (2710)
HpaI (2850)
EcoRI (2944)
SwaI (3200)


3201 AAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCCAGTTTAGTAGTTGGACTTAGGG
3301 AACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCC
125 ●●●AspGlnGluGluAlaValPheHisValCysAsnGlyAlaP
3401 GGGTCGCGCAGGGCGAACTCCCGCCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGGCCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACC
110 roAspArgLeuAlaPheGluArgGlyTrpProGlnGluGlyIleGluThrMetAlaProGlySerAlaAspArgPheAsnThrSerValValGluSerTr
3501 ACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACGTC
77 pGluAlaTyrLeuGluAspLeuGlyArgValTrpValTrpAlaLeuThrAsnAspProValValGlnAspGlnValAlaSerIlePheLeuThrValAsp
SmaI (3641)
3601 GTCCCGGACCACACCGGCGAAGTCGTCCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCGGTCCAGAACTCGACCGCTCCGGCGACGTCGCGCGCGGTG
44 AspArgValValGlyAlaPheAspAspGluValPheAspArgSerPheGlyLeuArgAspThrTrpPheGluValAlaGlyAlaValAspArgAlaThrL
AseI (3788)
3701 AGCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCCCTCCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAAA
10 euValProValAlaSerThrLeuLysAlaMet
3801 CAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTTCACTAAACGAGCTCTGCTTATATAGACCTCCCACCGTACACGCCTACCGCCCATTTGCGTCAATG
SpeI (3942)
3901 GGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTTACTAGTCAAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGA
4001 GTCAAACCGCTATCCACGCCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCC
4101 CATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCCA
4201 AGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCG
SdaI (4358) PacI (4365)
4301 GGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCA
4401 GGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCC
4501 GACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTC
4601 CCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCG
4701 TTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGAT
4801 TAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTG
4901 AAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGC
5001 GCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAG
PacI (5105) SwaI (5113) NotI (5121)
5101 TTAATTAACATTTAAATC AGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCC
5201 ATCAAAACAAAACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA