

pUNO1-hTMPRSS2a

Expression vector containing human TMPRSS2a open reading frame

Catalog code: puno1-htps2a

<https://www.invivogen.com/human-tmprss2-expression-vectors>

For research use only

Version 20F02-NJ

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

- After purification by ion exchange chromatography, predominant supercoiled conformation is verified by electrophoresis.
- Plasmid construct has been confirmed by restriction analysis and full-length open reading frame (ORF) sequencing.

GENERAL PRODUCT USE

- **Subclone gene into another vector.** Two unique restriction sites flank the gene, allowing convenient excision. The 5' site is AgeI which is compatible with XmaI, BspEI, NgoMIV, and SgrAI. The 3' site is NheI which is compatible with XbaI, 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.

PLASMID FEATURES

• human TMPRSS2a

ORF size: 1590 bp

Human TMPRSS2a (transmembrane protease serine 2, isoform 1) is a multimeric protein containing four domains, among which a type II transmembrane domain and a serine protease domain. It is widely expressed in epithelial tissues, including prostate, pancreas, liver, kidney, lung, colon, and small intestine¹. TMPRSS2 is capable of autoactivation, and its protease domain is thought to be secreted upon autocleavage². In the context of SARS-CoV-2 infection, TMPRSS2 exerts a crucial proteolytic activation of the S protein bound to ACE2 to facilitate the viral entry into target cells³⁻⁵. The hTMPRSS2 gene has two spliced transcript variants, hTMPRSS2a and hTMPRSS2b, encoding a long (1) and a short (2) isoform, respectively. The isoform 2 has an alternate 5' exon and uses a downstream AUG start codon, resulting in a shorter N-terminus as compared to the isoform 1. Both isoforms have been found to cleave the Spike protein of SARS-CoV for cathepsin L-independent entry into target cell⁶.

• **EF-1α/HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) core promoter⁷ 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 Repeat⁸ 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** is the Simian Virus 40 late polyadenylation (pAn) signal enables efficient cleavage and polyadenylation reactions, resulting in high levels of steady-state mRNA⁹.

TECHNICAL SUPPORT

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- **pMB1 ori** is a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.
- **hCMV (human cytomegalovirus) enhancer & promoter** drive the expression of the blasticidin resistance in mammalian cells.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **bsr (blasticidin resistance 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 pAn** is a strong polyadenylation (pAn) signal placed downstream of *bsr*. The use of beta-globin pAn minimizes interference¹⁰ and possible recombination events with the SV40 pAn signal.

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.

REFERENCES

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4. Hoffmann M. *et al.*, 2020. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell.* 181:1-16.
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9. Carswell S. & Alwine J., 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. *Mol Cell Biol.* 9(10):4248-58.
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RELATED PRODUCTS

| Product | Description | Cat. Code |
|--------------------|--------------------------|----------------|
| Blasticidin | Selection antibiotic | ant-bl-1 |
| ChemiComp GT116 | Competent <i>E. coli</i> | gt116-11 |
| pUNO1-hACE2 | Expression vector | puno1-hace2 |
| pUNO1-hTMPRSS2b | Expression vector | puno1-htps2b |
| pUNO1His-SARS2-S | Production vector | p1his-cov2-s |
| pUNO1Fc-SARS2-S | Production vector | p1fc-cov2-s |
| pUNO1His-SARS2-S1 | Production vector | p1his-cov2-s1 |
| pUNO1Fc-SARS2-S1 | Production vector | p1fc-cov2-s1 |
| pUNO1His-SARS2-RBD | Production vector | p1his-cov2-rbd |
| pUNO1Fc-SARS2-RBD | Production vector | p1fc-cov2-rbd |

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