pUNO1-hTMPRSS2b

Expression vector containing human TMPRSS2a open reading frame

Catalog code: puno1-htps2b

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 TMPRSS2b**

ORF size: 1479 bp

Human TMPRSS2b (transmembrane protease serine 2, isoform 2) 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 intestine[1]. TMPRSS2 is capable of autoactivation, and its protease domain is thought to be secreted upon autocleavage[2]. 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 cells[3-5]. 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 cells[6].

- **EF-1α/HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) core promoter[7] 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[8] 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 he Simian Virus 40 late polyadenylation (pAn) signal enables efficient cleavage and polyadenylation reactions, resulting in high levels of steady-state mRNA[9].

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 interference[10] 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

1. Bugge T.H. *et al.*, 2009. Type II transmembrane serine proteases. J. Bio. Chem. 284(35) :23177-23181. **2. Afar D.E.H, *et al.*, 2001.** Catalytic Cleavage of the Androgen-regulated TMPRSS2 Protease Results in Its Secretion by Prostate and Prostate Cancer Epithelia. Cancer Res. 61(4):1686-1692. **3. Li W. *et al.*, 2003.** Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 426(6965):450-454. **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. **5. Zhou P. *et al.*, 2020.** A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 579(7798):270-273. **6. Zmora P. *et al.*, 2015.** TMPRSS2 isoform 1 activates respiratory viruses and is expressed in viral target cells. PLoS ONE 10(9):e0138380. **7. Kim D. *et al.*, 1990.** Use of the human elongation factor 1α promoter as a versatile and efficient expression system. Gene 91(2):217-23. **8. 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. **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. **10. Yu J. & Russell J., 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.

RELATED PRODUCTS

Product	Description	Cat. Code
Blasticidin	Selection antibiotic	ant-bl-1
ChemiComp GT116	Competent *E. coli*	gt116-11
pUNO1-hACE2	Expression vector	puno1-hace2
pUNO1-hTMPRSS2a	Expression vector	puno1-htps2a
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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