pUNO1-SARS2-S-d19

Expression vector containing the truncated (Δ19) SARS-CoV-2 Spike (D614) open reading frame

Catalog code: puno1-cov2-sd19

https://www.invivogen.com/sars2-truncated-spike-expression-vector

For research use only

Version 20J05-ED

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 for at least 1 year.
- Store Blasticidin at 4°C or -20°C. The expiry date is specified on the product label.

Quality control

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

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.

PLASMID FEATURES

SARS-CoV-2 native D614 Spike (Δ19 truncated) cassette

- SARS-CoV-2 D614-Spike (Δ19 truncated) cassette ORF

Spike (S) is a structural glycoprotein expressed on the surface of SARS-CoV-2. It mediates membrane fusion and viral entry into target cells upon binding to the host receptor ACE2 and the proteolytic activity of TMPRSS2[1]. The S protein consists of an N-terminal ectodomain, a transmembrane anchor, and a short C-terminal cytoplasmic tail. The ectodomain contains the S1 subunit, which encodes the receptor binding domain (RBD), a key target in treatment and vaccination strategies against COVID-19, as well as the S2 subunit, needed for membrane fusion[2]. Notably, the C-terminal cytoplasmic tail of the S protein encodes a presumptive endoplasmic reticulum (ER)-retention motif (KxHxx), which has previously been shown to enable the accumulation of SARS-CoV S proteins at the ER-Golgi intermediate compartment (ERGIC) and facilitate their incorporation into new virions[3].

The pUNO1-SARS2-S-d19 plasmid contains the native D614 Spike coding sequence from the Wuhan-Hu-1 isolate. Furthermore, to improve expression of the S protein in pseudovirions and cell lines as reported in the literature, the last 19 amino acids (d19), which contain the ER-retention motif, have been removed[4,5].

- **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 a "house-keeping"gene. It is expressed at high levels in all cell cycles and lower levels during the G0 phase. Additionally, since the promoter is not 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.
- **SV40 pAn** is the Simian Virus 40 late polyadenylation (pAn) signal and it enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA[7].

Antibiotic selection cassette

- **hCMV (human cytomegalovirus) enhancer & promoter** drive the expression of the blasticidin resistance gene (*bsr*) in mammalian cells.
- **EM7** is a bacterial promoter that enables the constitutive expression of the blasticidin resistance gene (*bsr*) in *E. coli*.
- ***bsr* (blasticidin resistance gene)** encodes a deaminase from *Bacillus cereus* that confers resistance to the antibiotic blasticidin. The expression of the *bsr* gene is driven by the CMV promoter/enhancer and the bacterial EM7 promoter. Therefore, Blasticidin can be used to select stable clones in mammalian cells and *E. coli* transformants.
- **Human β-Globin pAn** is a strong polyadenylation (pAn) signal placed downstream of *bsr*. The use of β-globin pAn minimizes interference and possible recombination events with the SV40 pAn signal[9].

General features of pUNO1-SARS2-S-d19

- **pMB1 ori** is a minimal *E. coli* origin of replication.

REFERENCES

1. 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. **2. Walls A.C., *et al.*, 2020.** Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell. 181(2):281-292.e6. **3. Ujike, M. *et al.* 2016.** The contribution of the cytoplasmic retrieval signal of severe acute respiratory syndrome coronavirus to intracellular accumulation of S proteins and incorporation of S protein into virus-like particles. J Gen Virol 97, 1853-1864. **4. Johnson, M.C. *et al.* 2020.** Optimized pseudotyping conditions for the SARS-COV2 Spike glycoprotein. bioRxiv. **5. Ou, X. *et al.* 2020.** Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun 11, 1620. **6. 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 **7. 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. **8. 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. **9. 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.

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 3622-3480

E-mail: info@invivogen.com

GENERAL PRODUCT USE

- **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 plasmids faciliate high levels of expression and secretion of the gene product.
- **Subclone gene into another vector.** Unique restriction sites flank the SARS-CoV-2 S (Δ19) gene allowing convenient excision.

5' - **AgeI** which is compatible with XmaI, BspEI, NgoMIV, and SgrAI.
3' - **NheI** which is compatible with XbaI, SpeI, and AvrII.

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-htp2a
pUNO1-hTMPRSS2b	Expression vector	puno1-htp2b

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 3622-3480
E-mail: info@invivogen.com