

pUNO1-SARS2-S-d19 (D614G)

Expression vector containing the truncated ($\Delta 19$) SARS-CoV-2 Spike G614-variant open reading frame

Catalog code: puno1-cov2-sd19g

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

For research use only

Version 20J05-ED

PRODUCT INFORMATION

Contents

- 20 μ g 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 $1\ \mu\text{g}/\mu\text{l}$, 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 DH5 α .

• Blasticidin usage

Blasticidin should be used at 25-100 $\mu\text{g}/\text{ml}$ in bacteria and 1-30 $\mu\text{g}/\text{ml}$ in mammalian cells. Blasticidin is supplied as a 10 mg/ml colorless solution in HEPES buffer.

PLASMID FEATURES

SARS-CoV-2 Spike G614-variant ($\Delta 19$ truncated) cassette

• SARS-CoV-2 G614-Spike ($\Delta 19$ 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 TMPRSS2¹. 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 fusion². 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 virions³.

The pUNO1 SARS2 S-d19 (D614G) plasmid encodes the globally dominant spike G614-variant⁴. 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-1 α /HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1 α (EF-1 α) core promoter⁷ and the 5' untranslated region of the Human T-Cell Leukemia Virus (HTLV). EF-1 α 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 Repeat⁸ has been coupled to the EF-1 α 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 mRNA⁷.

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 signal⁹.

General features of pUNO1-SARS2-S-d19 (D614G)

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

REFERENCES

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TECHNICAL SUPPORT

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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 facilitate high levels of expression and secretion of the gene product.

- **Subclone gene into another vector.** Unique restriction sites flank the SARS-CoV-2 S (Δ 19) 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 <i>E. coli</i>	gt116-11
pUNO1-SARS2-S	Expression vector	puno1-cov2-s
pUNO1-hACE2	Expression vector	puno1-hace2
pUNO1-hTMPRSS2a	Expression vector	puno1-htp2a
pUNO1-hTMPRSS2b	Expression vector	puno1-htp2b

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