**pUNO1-SARS2-S (D614G)**

Expression vector containing SARS-CoV-2 Spike (D614G) open reading frame

Catalog code: puno1-cov2-sg

[https://www.invivogen.com/sars2-full-spike-expression-vector](https://www.invivogen.com/sars2-full-spike-expression-vector)

For research use only

Version 20H12-NJ

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**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 is 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**

- SARS-CoV-2 (D614G) full Spike
  - **ORF size**: 3822 bp
  - Spikes are multifunctional glycoproteins that mediate the entry of coronaviruses into the target cell and are critical determinants of the viral host and tissue tropism. Spikes exhibit a large ectodomain comprised of two subunits. The S1 subunit contains the ACE2 receptor binding domain (RBD), while the S2 subunit features the elements mediating the fusion of viral and host membranes. Protein vaccination studies using the full Spike or its S1 or RBD fragments have provided encouraging results to protect from SARS-CoV and MERS-CoV.

- **EF-1α/HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) core promoter 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 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 that enables efficient cleavage and polyadenylation reactions, resulting in high levels of steady-state mRNA.

- **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 and possible recombination events with the SV40 pAn signal.

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**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

[www.invivogen.com](http://www.invivogen.com)
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


RELATED PRODUCTS

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