

pUNO1-SpikeV16

Expression vector encoding the SARS-CoV-2 Omicron variant (JN.1 lineage) Spike gene

Catalog code: p1-spike-v16

<https://www.invivogen.com/omicron-jn1-spike-expression-vectors>

For research use only

Version 24G30-AK

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized pUNO1-SpikeV16 (plasmid DNA)
- 2 x 1 ml of **Blasticidin** (10 mg/ml)

Storage and Stability

- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20°C.
- Resuspended DNA is stable for 1 year at -20°C.
- 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.

PLASMID FEATURES

Omicron Variant (JN.1 lineage) SARS-CoV-2 Spike cassette

- **EF-1α/HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) core promoter¹ 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 Repeat² 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.

- **Codon-optimized Spike ORF**

pUNO1-SpikeV16 contains the Spike coding sequence from the Omicron SARS-CoV-2 variant (JN.1 lineage), first identified in the USA in September 2023. This variant is characterized by several of mutations and deletions within the Spike coding sequence (see below)^{3,4}. Additionally, to improve expression of the S protein in cell lines, the gene is codon-optimized and the last 19 amino acids, which contain an endoplasmic reticulum (ER)-retention motif (KxHxx), have been removed^{5,6}.

pUNO1-SpikeV16 includes the following sequence features:

- **S1 domain:** ins16MPLF, T19I, R21T, deletion (Δ)L24-P26, A27S, S50L, ΔH69-70, V127F, G142D, ΔY144, F157S, R158G, ΔN211, L212I, V213G, L216F, H245N, A264D, E554K, A570V, D614G, P621S, H655Y, N679K, P681R
- **RBD:** I332V, G339H, K356T, S371F, S373P, S375F, T376A, R403K, D405N, R408S, K417N, N440K, K445H, G446S, N450D, L452W, L455S, N460K, S477N, T478K, N481K, ΔV483, E484K, F486P, Q498R, N501Y, Y505H
- **S1/S2 boundary:** Functional furin cleavage site
- **S2 domain:** N764K, D796Y, S939F, Q954H, N969K, P1143L

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 host proteases such as furin and TMPRSS2⁷.

- **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 mRNA⁸.

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-SpikeV16

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

APPLICATIONS

Stable gene expression in mammalian cells.

pUNO1 plasmids are designed for both transient and stable transfection in mammalian cell lines by selection with **Blasticidin**. Furthermore, they facilitate high levels of expression of the gene of interest.

Cell fusion assays

pUNO1-SpikeV16 has been specifically designed for mammalian cell expression of the SARS-CoV-2 S protein. This plasmid features a functional furin cleavage site, and therefore when expressed by a host cell (e.g. 293T cells) it facilitates cell-cell fusion assays with ACE2-expressing cells (e.g. **HEK-Blue™ hACE2 Cells**)¹⁰.

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 the 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.

TECHNICAL SUPPORT

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REFERENCES

1. Kim D. *et al.*, 1990. Use of the human elongation factor 1 α promoter as a versatile and efficient expression system. *Gene* 91(2):217-23 2. 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. 3. <https://www.who.int/en/activities/tracking-SARS-CoV-2-variants>. 4. <https://outbreak.info/situation-reports>. 5. Johnson, M.C. *et al.* 2020. Optimized Pseudotyping Conditions for the SARS-COV-2 Spike Glycoprotein. *J Virol* 94. 6. 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. 7. 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. 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. 10. Papa, G. *et al.* 2021. Furin cleavage of SARS-CoV-2 Spike promotes but is not essential for infection and cell-cell fusion. *PLoS Pathog* 17, e1009246.

RELATED PRODUCTS

Product	Description	Cat. Code
Blasticidin	Selection antibiotic	ant-bl-1
ChemiComp GT116	Competent <i>E. coli</i>	gt116-11
COVID-19 Product Range		
293-hMyD88 Cells	Cell line	293-hmyd
HEK-Blue™ hACE2 Cells	Cell line	hkb-hace2
A549-hACE2-TMPRSS2 Cells	Cell Line	a549-hace2-tpsa
pUNO1-hACE2	Expression vector	puno1-hace2
pUNO1-hTMPRSS2a	Expression vector	puno1-htp2a
Anti-CoV2RBD-cas-hlgG1	Recombinant Antibody	srbdc3-mab1

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