

# pUNO1-SpikeV4

Expression vector encoding the SARS-CoV-2 Californian variant (B.1.429 lineage) Spike gene

Catalog code: p1-spike-v4

<https://www.invivogen.com/cal-b1429-spike-expression-vectors>

For research use only

Version 21C22-ED

## PRODUCT INFORMATION

### Contents

- 20 µg of lyophilized pUNO1-SpikeV4 (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

### Californian Variant SARS-CoV-2 Spike cassette

• **EF-1α/HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) core promoter<sup>1</sup> 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<sup>2</sup> 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-SpikeV4 contains the Spike coding sequence from the Californian (CAL) SARS-CoV-2 variant (B.1.429 lineage). This variant is characterized by a number of deletions (del) and mutations within the the Spike coding sequence (see below)<sup>3</sup>. 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<sup>4,5</sup>.

pUNO1-SpikeV4 includes the following sequence features:

- **S1 domain:** S13I, W152C, D614G
- **RBD:** L452R
- **S1/S2 boundary:** Functional furin cleavage site

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 TMPRSS2<sup>6</sup>.

For more information visit: <https://www.invivogen.com/sars2-spike>

- **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<sup>7</sup>.

### 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<sup>8</sup>.

### General features of pUNO1-SpikeV4

- **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-SpikeV4 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**)<sup>9</sup>.

## 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 $\alpha$  promoter as a versatile and efficient expression system. *Gene* 91(2):217-23

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8. Yu J. & Russell J., 2001. Structural and functional analysis of an mRNP complex that mediates the high stability of human  $\beta$ -globin mRNA. *Mol Cell Biol.* 21(17):5879-88.

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## RELATED PRODUCTS

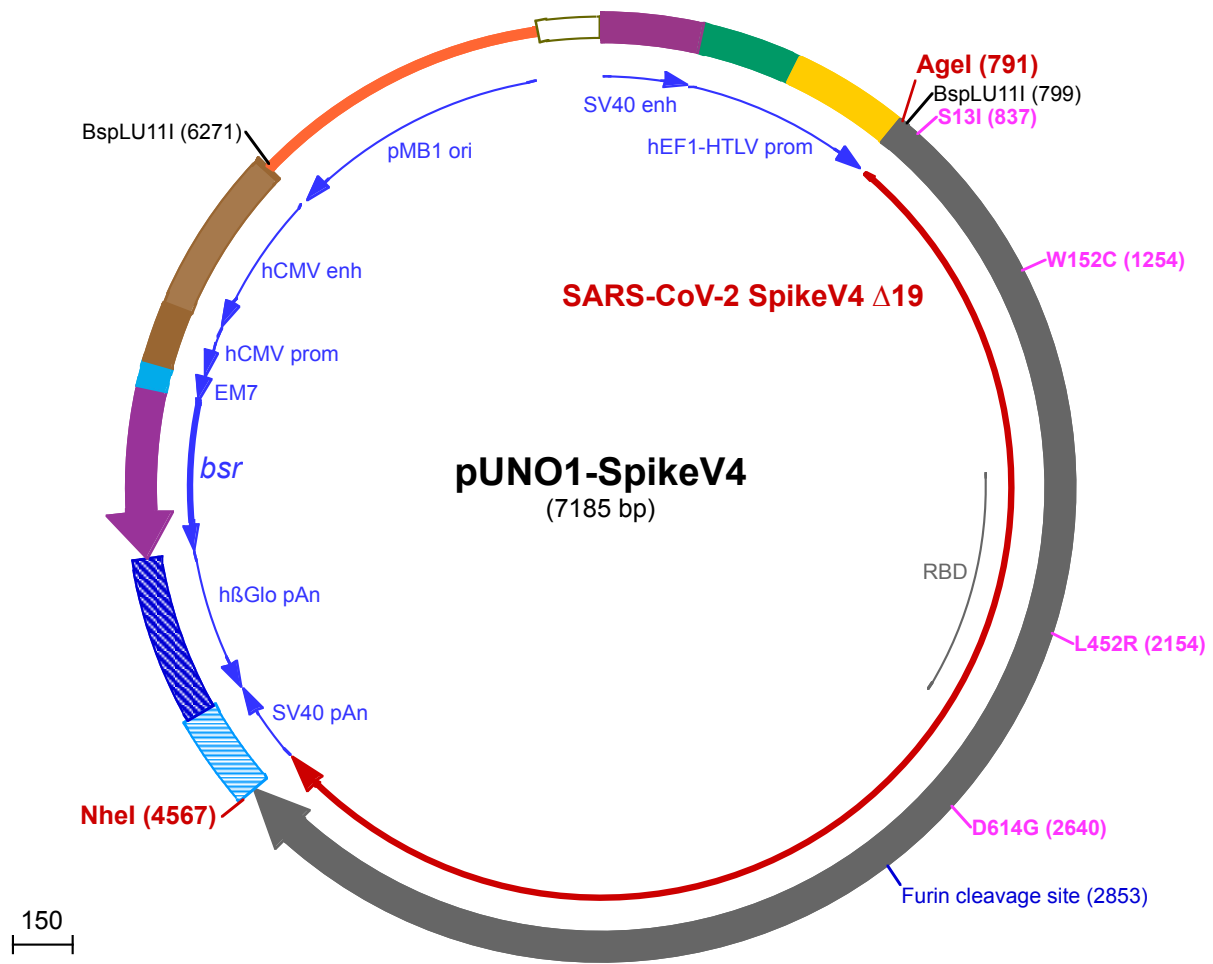
Product	Description	Cat. Code
Blasticidin	Selection antibiotic	ant-bl-1
ChemiComp GT116	Competent <i>E. coli</i>	gt116-11
<b>COVID-19 Product Range</b>		
HEK-Blue™ hACE2 Cells	Cell line	hkb-hace2
A549-hACE2-TMPRSS2 Cells	Cell Line	a549-hace2-tpsa
pUNO1-hACE2	Expression vector	puno1-hace2
pUNO1-hTMPRSS2a	Expression vector	puno1-htp2a
Anti-CoV2RBD-c1-hlgG1	Recombinant Antibody	cov2rbdc1-mab1

For a complete list of InvivoGen's COVID-19 related products visit: <https://www.invivogen.com/covid-19>

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1 GGACCTGCAGGGCCTGAAATAACCTCTGAAAGAGGAACCTTGGTTAGGTACCTTCTGAGGCGGAAAGAACCAGCTGTGGAATGTGTGTAGTTAGGGTGTG  
 101 GAAAGTCCCAGGCTCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCAGGCTCCCAGCAGGCAG  
 201 AAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCACTAGTCTCGGTGCCCGTCACTGGGCGAGCGCACATCGCCACAGTCCC  
 301 GAAGTTGGGGGAGGGGTGCGCAATTGAACGGGTGCCTAGAGAAGGTGGCGGGGTAACAGTGGGAAAGTGTGCTGTACTGGCTCCGCTTTTTCC  
 401 GAGGGTGGGGGAGAACCGTATATAAGTCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTCCGCCAGAACAGCTGAAGCTTCGAGGGGCTC  
 501 GCATCTCTCTTACGCGCCCGCCCTACCTGAGGCCGCATCCACGCCGGTTGAGTCGCGTTCTGCCGCTCCCGCTGTGGTGCCTCCTGAAGTGC  
 601 GTCCGCGCTTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGCGCTCCCTTGAGGCTACCTAGACTCAGCCGGCTCTCCACGCTTGC

Agel (791)

701 CTGACCCTGCTTGCTCAACTCTACGCTTTTGTTCGTTTTCTGTTCTGCGCAGTTACAGATCCAAGCTGTGACCGCGCCTACCTGAGATCACCGGTCAA

S131 (837)

801 CATGTTTGTGTTCTTGGTGTGCTTCCACTGGTCAGTATCAATGCGTAACTCTACCACCCGAACTCAACTCCCACCCGCATATACAAATTCCTTACC  
 1 M F V F L V L L P L V S I Q C V N L T T R T Q L P P A Y T N S F T  
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 34 R G V Y Y P D K V F R S S V L H S T Q D L F L P F F S N V T W F H  
 1001 CAATCCATGTGTCTGGGACAAACGGCACAAACGCTTCCGACAACTGATTTGCCATTCAATGATGGGGTGTACTTTGCCTCCACAGAGAAATCCAACAT  
 67 A I H V S G T N G T K R F D N P V L P F N D G V Y F A S T E K S N I  
 1101 CATTGAGGATGGATTTTCGGGACTACTCTGACTCAAAGACACAGAGCCTGCTGATCTTAACAACGCCACAAACGTTGTATCAAAGTGTGCGAATTC  
 100 I R G W I F G T T L D S K T Q S L L I V N N A T N V V I K V C E F

W152C (1254)

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 1301 CCTTCGAGTACGTGAGTCAACCTTTCTGATGGACCTGGAAGGAAACAGGGAAACTTCAAGAACCTGAGAGAGTTTGTCTTTAAGAACATCGACGGCTA  
 167 T F E Y V S Q P F L M D L E G K Q G N F K N L R E F V F K N I D G Y  
 1401 TTTAAGATCTATAGTAAGCATACGCCATCAACCTGGTAAGGGATCTTCCCAAGGCTTTTCCAGCCTGGAACCTTTGGTTGACTTGCCTATTGGTATC  
 200 F K I Y S K H T P I N L V R D L P Q G F S A L E P L V D L P I G I  
 1501 AATATCACCAGATTTCCAGACCTTCTGGCATTGCATCGGTCTTACTTCCAGGTGATTCCTCCTCCGGGTGGACTGCCGGCGCCGCTGCCTACTATG  
 234 N I T R F Q T L L A L H R S Y L T P G D S S S G W T A G A A A Y Y  
 1601 TCGGCTATCTGCAACCAAGAAGTTCCTGCTCAAGTACAACGAAACGGCACTATTACGGATGCTGTTGATTGTGCCCTGGACCCTCTGTCTGAGACTAA  
 267 V G Y L Q P R T F L L K Y N E N G T I T D A V D C A L D P L S E T K  
 1701 ATGCACCCTCAAGAGCTTTACCGTTGAGAAGGGGATTTACCAAAACAGTAATTTCCGGGTCCAACCCAGAAAGCATTGTGCGGTTCCCAATATCACC  
 300 C T L K S F T V E K G I Y Q T S N F R V Q P T E S I V R F P N I T

1801 AATCTGTGTCCTTTGGCGAAGTGTCAATGCTACAAGTTTGTCTGTGTACGCATGGAATAGGAAACGCATCTCCAATTGTGTGCTGATTACTCCG  
 334 N L C P F G E V F N A T R F A S V Y A W N R K R I S N C V A D Y S

1901 TGCTGTACAATCCGCTCTTTCTCAACCTTCAAGTGTATGGCGTTTACCTACCAAACTTAAACGACCTGTGCTTCACTAATGTGTATGCCGACTCTTT  
 367 V L Y N S A S F S T F K C Y G V S P T K L N D L C F T N V Y A D S F

2001 TGTGATACGAGGCGATGAAGTGAACAGATTGACCAGGGCAGACCGGCAAAATGCGGACTACAACATAAGCTTCCAGATGACTTTACCGGATGTGTT  
 400 V I R G D E V R Q I A P G Q T G K I A D Y N Y K L P D D F T G C V

L452R (2154)

2101 ATTGCATGGAACCTCAACAATCTGGATTCCAAGGTGGGTGGCAACTATAACTACCGCTATAGACTGTTCCAGGAAATCCAACCTGAAACCATTCGAGCGAG  
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2201 ATATAAGCACAGAAATCTACCAGGCTGGAAGTACGCCCTGCAACGGCGTGGAAAGGTTCAACTGCTACTTCCATTGCAAGTACCGGATTCCAGCCTAC  
 467 D I S T E I Y Q A G S T P C N G V E G F N C Y F P L Q S Y G F Q P T

2301 AAACGGGTGGGTTACCAACCTATCGTGTCTGAGTCTTGGAGTCTTCCATGCCCCAGCCACAGTCTGTGGCCCCAAGAAAAGCACAATCTG  
 500 N G V G Y Q P Y R V V V L S F E L L H A P A T V C G P K K S T N L

2401 GTGAAGAACAATGCGTGAACCTTAACTTTAACGGACTCACAGGAACCGCGTATTGACGGAGAGTAACAAGAAGTCTGCCATTCCAGCAGTTCGGTC  
 534 V K N K C V N F N F N G L T G T G V L T E S N K K F L P F Q Q F G

2501 GCGATATTGCCGACACTACCGACGCTGTCCGAGATCCCCAGACATTGGAGATTCTTGATATCACACCCTGTAGTTTCCGGCGGAGTGAGCGTGATTACGCC  
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D614G (2640)

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2701 CGAGTATATCCACCGGCTCCAAAGTCTTTTCAGACACGTGCTGGATGTCTGATCGGTGCAGAACACGTTAATAATAGCTACGAGTGTGATATCCCCATCG  
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700▶ A E N S V A Y S N N S I A I P T N F T I S V T T E I L P V S M T K  
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1167▶ G D I S G I N A S V V N I Q K E I D R L N E V A K N L N E S L I D L  
4401 GCAGGAGTTGGCAAGTACGAACAGTATATCAAATGGCCATGGTACATTTGGCTTGGGTTCAATGCTGGGCTGATAGCTATCGTCATGGTGAACATATG  
1200▶ Q E L G K Y E Q Y I K W P W Y I W L G F I A G L I A I V M V T I M

**NheI (4567)**

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6001 CCATAAGGTCATGTA CTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTA CTGCC  
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6201 GGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAAGTTATGTAACGCCTGCAGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCC  
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