

# pUNO1-SpikeV1-dfur

Expression vector containing the SARS-CoV-2 Wuhan-Hu-1 G614-variant Spike (delta furin) gene

Catalog code: p1-spike-v1-df

<https://www.invivogen.com/wuhan-spike-d614g-expression-vectors>

For research use only

Version 21C15-ED

## PRODUCT INFORMATION

### Contents

- 20 µg of lyophilized pUNO1-SpikeV1-dfur (plasmid DNA)
- 2 x 1 ml of **Blasticidin** (10 mg/ml)

### Storage and Stability

- Product is shipped at room temperature.
- Store lyophilized DNA 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

### Wuhan-Hu-1 SARS-CoV-2 Spike (G614) cassette

• **EF-1α/HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) core promoter<sup>3</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-SpikeV1-dfur contains the Spike (S) coding sequence from the Wuhan-Hu-1 G614-variant, characterized by the D614G mutation<sup>4</sup>. This signature mutation has globally replaced the original sequence and is present in all subsequent variants of SARS-CoV-2<sup>3</sup>. The furin cleavage site in pUNO1-SpikeV1-dfur has been inactivated (dfur) by the inclusion of two mutations (R683/5A). Furthermore, 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-SpikeV1-dfur includes the following sequence features:

- **S1 domain:** D614G
- **S1/S2 boundary:** R683A, R685A.

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-SpikeV1-dfur

- **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.

### Antibody screening by flow cytometry

pUNO1-SpikeV1-dfur has been specifically designed for mammalian cell expression of the SARS-CoV-2 S protein. Notably, due to the inactivated furin cleavage site, when this plasmid is expressed by a host cell (e.g. 293T cells) there is high surface expression of the full-length S protein<sup>4,9</sup>. Ideal for SARS-CoV-2 S-specific antibody screening by flow cytometry (*in-house data*).

## 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.

## TECHNICAL SUPPORT

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## REFERENCES

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## RELATED PRODUCTS

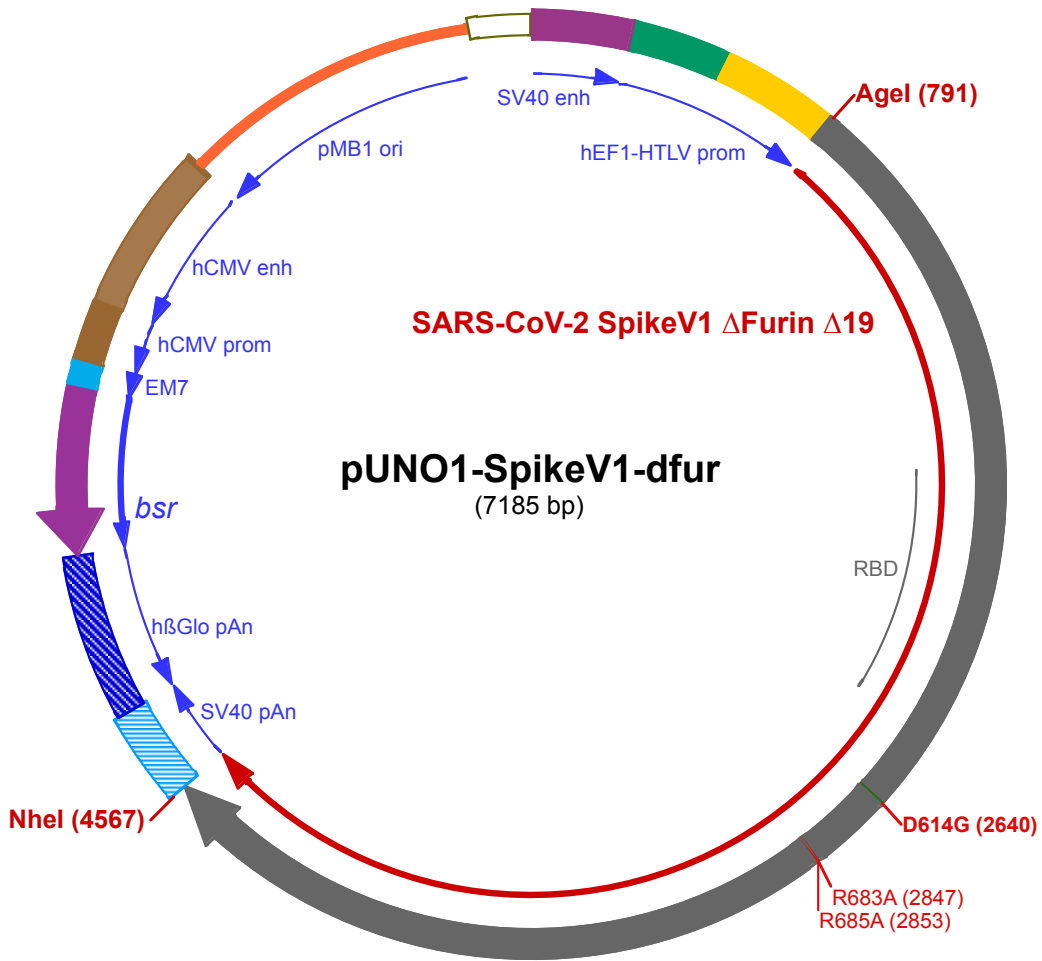
Product	Description	Cat. Code
Blasticidin	Selection antibiotic	ant-bl-1
ChemiComp GT116	Competent <i>E. coli</i>	gt116-11
<b>COVID-19 Product Range</b>		
HEK-Blue™ hACE2 Cells	Cell line	hkb-hace2
A549-hACE2-TMPRSS2 Cells	Cell Line	a549-hace2-tpsa
pUNO1-hACE2	Expression vector	puno1-hace2
pUNO1-hTMPRSS2a	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 GGACCTGCAGGGCCTGAATAACCTCTGAAAGAGGAACCTGGTTAGGTACCTTCTGAGCGGAAAGAACAGCTGTGGAATGTGTGTCAGTTAGGGTGTG  
101 GAAAGTCCCAGGCTCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAGTCCCAGGCTCCCAGCAGGCAG  
201 AAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCACTAGTCTCCGGTGGCCGTGAGTGGGAGAGCGCACATCGCCACAGTCCCCGA  
301 GAAGTTGGGGGAGGGGTGCGCAATTGAACGGGTGCCTAGAGAAGGTGGCGGGGTAACCTGGGAAAGTGTGCTGTACTGGCTCCGCTTTTTCC  
401 GAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGGCCGAGAACACAGCTGAAGCTTCGAGGGGCTC  
501 GCATCTCTCTTACGCGCCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCTCCCGCTGTGGTGCCTCCTGAACTGC  
601 GTCCGCGCTTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGC

Agel (791)

701 CTGACCCTGCTTGTCAACTCTACGTCTTTGTTTCTGTTTCTGCGCAGTTACAGATCCAAGCTGTGACCGGCGCTACCTGAGATCACCGTCAA  
801 CATGTTTGTGTTCTTGGTGTGCTTCCACTGGTCAGTCCCAATGCGTAAATCTACCACCCGAACCTCAACTCCCACCCGCATATACAAATCCTTCACC  
1 M F V F L V L L P L V S S Q C V N L T T R T Q L P P A Y T N S F T  
901 AGAGGAGTGTACTATCCTGACAAAGTGTTCGGTCAAGTGCCTCCACTCTACTCAGGACCTCTTCTGCCTTTCTTTTCTAACGTTACATGGTTTCATG  
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 CAATCCATGTGTCTGGGACAAACCGCACAAACGCTTCGACAACCCTGTATTGCCATTCAATGATGGGGTGTACTTTGCTCCACAGAGAAATCCAACAT  
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 CATTGAGGATGGATTTTCGGGACTACTCTGGACTCAAAGACACAGAGCCTGTGATCGTTAAACAACGCCACAAACGTTGTCATCAAAGTGTGCGAATTC  
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  
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1401 TTTAAGATCTATAGTAAGCATAACGCTATCAACCTGGTAAGGGATCTTCCCAGGGCTTTTCAGCCCTGGAACCTTTGGTTGACTTGCCTATTGGTATC  
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1601 TCGGCTATCTGCAACCAAGAAGTTCCTGCTCAAGTACAACGAAAACGGCACTATTACGGATGCTGTTGATTGTGCCCTGGACCTCTGTCTGAGACTAA  
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  
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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 TGCTGTACAATTCGCTCTTTCTCAACCTTCAAGTGTATGGCGTTTACCTACCAAACCTAACGACCTGTGCTTACTAATGTGTATGCCGACTCTTT  
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 TGTGATACGAGGGCATGAAGTGAAGCAGATTGCACCAGGGCAGACCGGCAAAATTCGCCGACTACAACATAAGCTTCCAGATGACTTTACCGGATGTGTT  
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  
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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  
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2401 GTGAAGAACAATGCGTGAACCTTAACTTAAACGGACTCACAGGAACCGCGTATTGACGGAGAGTAACAAGAAGTCTGCTGCACTTCCAGCAGTTCCGGTC  
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 GCGATATTGCCGACACTACCGACGCTGTCCGAGATCCCAGACATTGGAGATTCTTGATATCACACCCTGTAGTTTCCGGCGGAGTGAGCGTGATTACGCC  
567 R D I A D T T D A V R D P Q T L E I L D I T P C S F G G V S V I T P  
D614G (2640)  
2601 CGGAACCAATACCAGCAATCAGGTTGCCGTCTGTATCAGGCGTGAATTGCACCGAGGTACCTGTGCCATCCAGCTGACCAACTTACCCACATGG  
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634 R V Y S T G S N V F Q T R A G C L I G A E H V N N S Y E C D I P I

R685A (2853)

R683A (2847)

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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 GCAGGAGTTGGGCAAGTACGAACAGTATATCAAATGGCCATGGTACATTTGGCTTGGGTTCTTGTGCTGGGCTGATAGCTATCGTCATGGTGAACAATTATG  
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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141▶ • N R T Y K L P I L E E I T T K V L K G N M E I L V F C D P  
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