

pUNO1-SpikeV4-dfur

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

Catalog code: p1-spike-v4-df

<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-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

Californian (CAL.) SARS-CoV-2 variant Spike cassette

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

- **Codon-optimized Spike ORF**

pUNO1-SpikeV4-dfur contains the Spike (S) coding sequence from the Californian (CAL.) 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)³. The furin cleavage site in pUNO1-SpikeV4-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 ER-retention motif (KxHxx), have been removed^{4,5}.

pUNO1-SpikeV3-dfur includes the following sequence features:

- **S1 domain:** L18F, D80A, D215G, Del-242-244, D614G
- **RBD:** K417N, E484K, N501Y
- **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 TMPRSS2⁶.

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 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 signal⁸.

General features of pUNO1-SpikeV4-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-SpikeV4-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^{4,9}. 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 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 DH5 α .

• 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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RELATED PRODUCTS

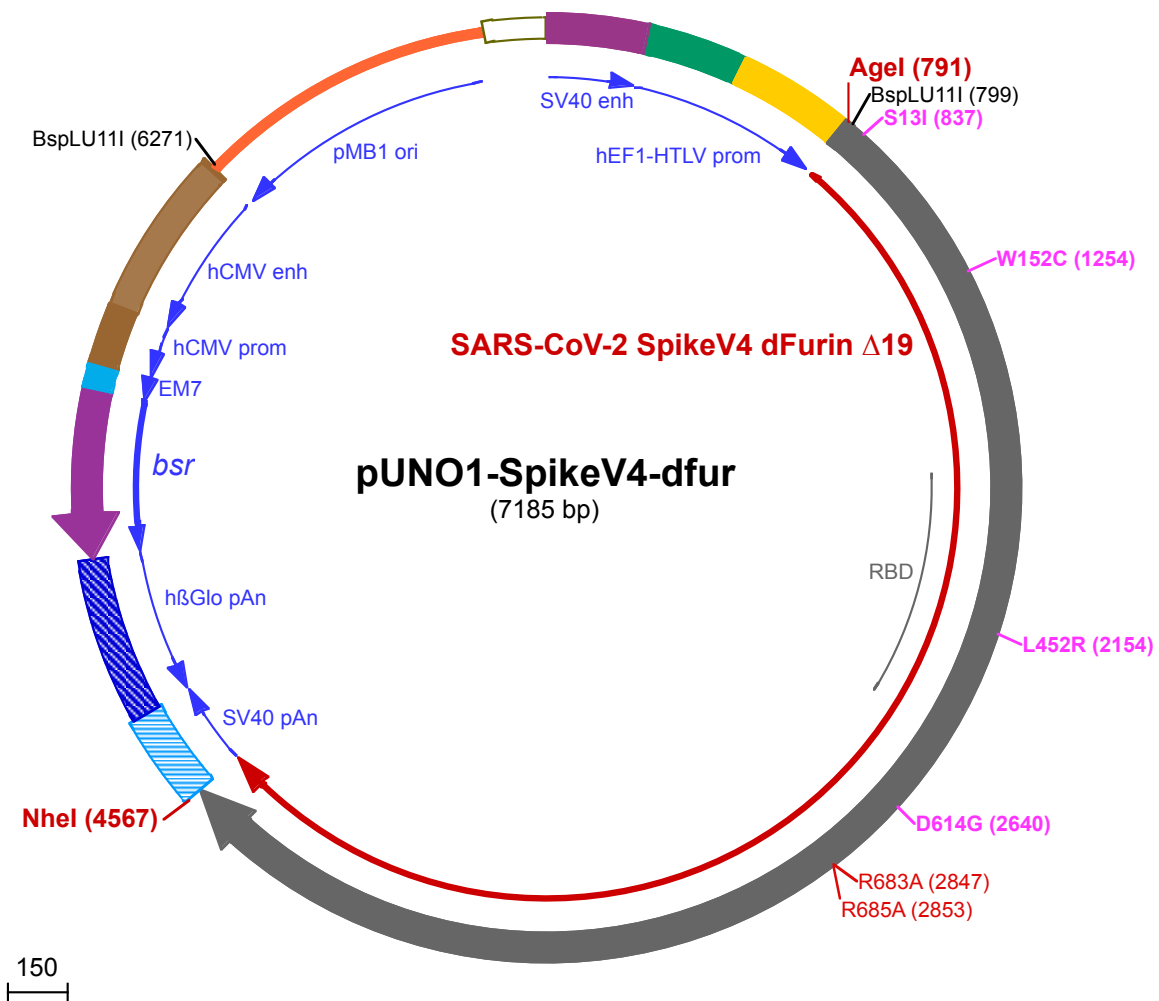
Product	Description	Cat. Code
Blasticidin	Selection antibiotic	ant-bl-1
ChemiComp GT116	Competent <i>E. coli</i>	gt116-11
COVID-19 Product Range		
HEK-Blue™ hACE2 Cells	Cell line	hkb-hace2
A549-hACE2-TMPRSS2 Cells	Cell Line	a549-hace2-tpsa
pUNO1-hACE2	Expression vector	puno1-hace2
pUNO1-hTMPRSS2a	Expression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

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1 GGACCTGCAGGGCCTGAAATAACCTCTGAAAGAGGAACTTGGTTAGGTACCTTCTGAGGCGGAAAGAACCAGCTGTGGAATGTGTGTAGTTAGGGTGTG
 101 GAAAGTCCCAGGCTCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCAGGCTCCCAGCAGGCAG
 201 AAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCACTAGTCTCGGTGCCCGTCACTGGGCGAGCGCACATCGCCACAGTCCC
 301 GAAGTTGGGGGAGGGGTGCGCAATTGAACGGGTGCCTAGAGAAGGTGGCGGGGTAACAGTGGGAAAGTGTCTGTACTGGCTCCGCTTTTTCC
 401 GAGGGTGGGGGAGAACCGTATATAAGTCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTCCGCCAGAACAGCTGAAGCTTCGAGGGGCTC
 501 GCATCTCTCTTACGCGCCCGCCCTACCTGAGGCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCTCCCGCTGTGGTGCCTCCTGAAGTGC
 601 GTCCGCGCTTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCTTTGTCCGCGCTCCCTTGAGGCTACCTAGACTCAGCCGGCTCTCCACGCTTTC

Agel (791)

701 CTGACCCTGCTTGCTCAACTCTACGCTTTTGTTCGTTTTCTGTTCTGCGCAGTTACAGATCCAAGCTGTGACCGCGCCTACCTGAGATCACCGTCAA

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 CAATCCATGTGTCTGGGACAAACGGCACAAACGCTTCCGACAACTGATTGCCATTCAATGATGGGGTGTACTTTGCCTCCACAGAGAAATCCAACAT
 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
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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 AATATCACCAGATTTCCAGACCTTCTGGCATTGCATCGGTCTTACTTCCAGGTGATTCTCCTCCGGGTGGACTGCCGGCGCCGCTGCCTACTATG
 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 ATGCACCCTCAAGAGCTTTACCGTTGAGAAGGGGATTTACCAAAACAGTAATTTCCGGGTCCAACCCAGAAAGCATTGTGCGGTTCCCAATATACC
 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 TGCTGTACAATCCGCTCTTTCTCAACCTTCAAGTGTATGGCGTTTCCACTACCAAACTTAAACGACCTGTGCTTCACTAATGTGTATGCCGACTCTTT
 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 TGTGATACGAGGCGATGAAGTGAACAGATTGACCAGGGCAGACCGGCAAAATTGCCGACTACAACATAAGCTTCCAGATGACTTTACCGGATGTGTT
 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
 434 I A W N S N N L D S K V G G N Y N Y R Y R L F R K S N L K P F E R

2201 ATATAAGCACAGAAATCTACCAGGCTGGAAGTACGCCCTGCAACGGCGTGGAAAGGTTCAACTGCTACTTCCATTGAGAGTTACGGATTCCAGCCTAC
 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 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 GCGATATTGCCGACACTACCGACGCTGTCCGAGATCCCCAGACATTGGAGATTCTTGATATCACACCCTGTAGTTTCGGCGGAGTGAGCGTGATTACGCC
 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)

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R685A (2853)
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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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NheI (4567)
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1234▶ L C C M T S C C S C L K G C C S C G S C C •
4601 GAGTTTGACAAAACCACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATA
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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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