

pUNO1-SpikeV5-dfur

Expression vector encoding the SARS-CoV-2 Brazilian variant (P.1 lineage) Spike (delta furin) gene

Catalog code: p1-spike-v5-df

<https://www.invivogen.com/brazil-p1-spike-expression-vectors>

For research use only

Version 21E07-ED

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized pUNO1-SpikeV5-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

Brazilian Variant 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-SpikeV5-dfur contains the Spike (S) coding sequence from the Brazilian (BRA.) variant (P.1 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-SpikeV5-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-SpikeV5-dfur includes the following sequence features:

- **S1 domain:** L18F, T20N, P26S, D138Y, R190S, D614G, H655Y
- **RBD:** K417T, E484K, N501Y
- **S1/S2 boundary:** R683A, R685A
- **S2 domain:** T1072I, V1176F

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-SpikeV5-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-SpikeV5-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 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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RELATED PRODUCTS

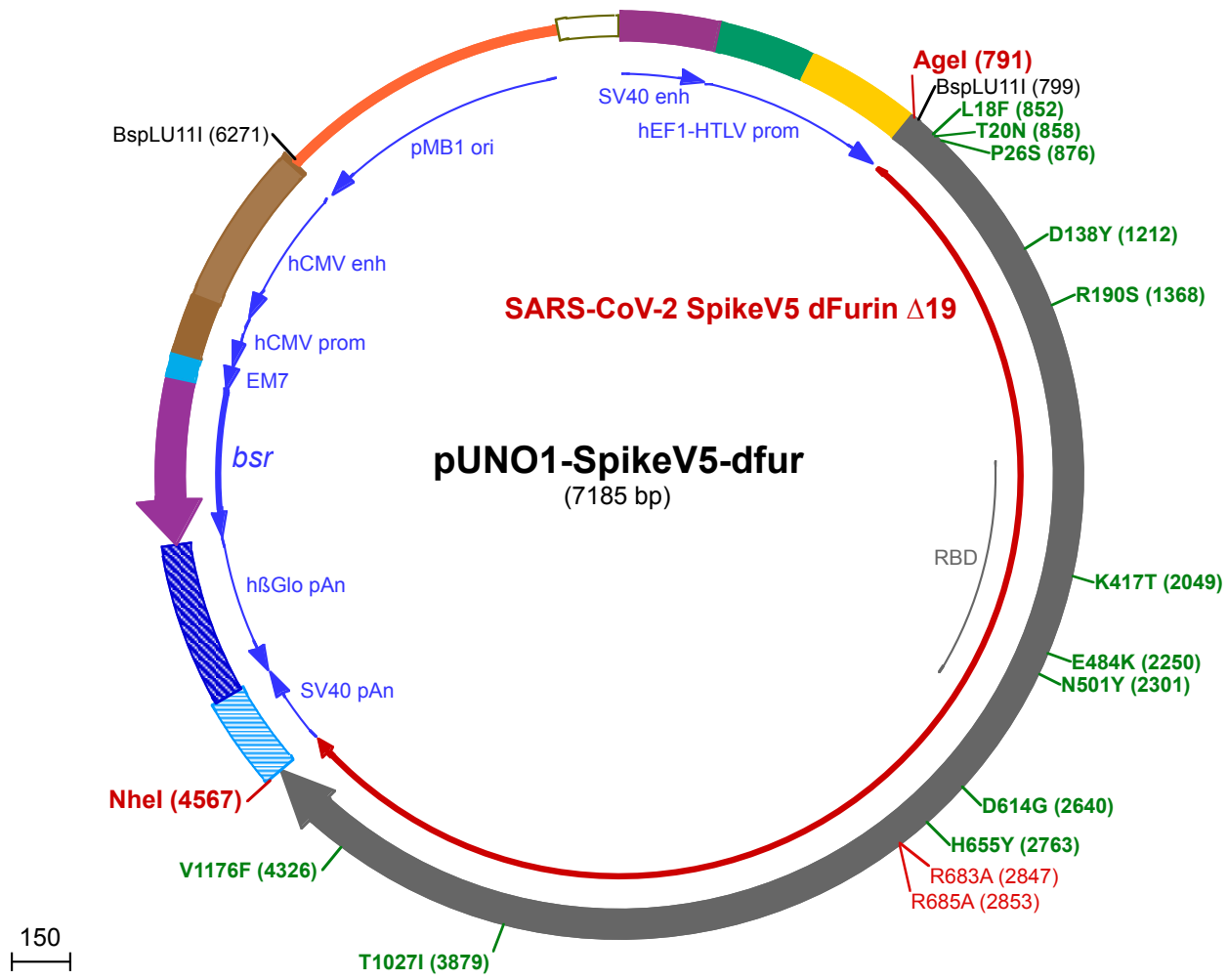
Product	Description	Cat. Code
Blasticidin	Selection antibiotic	ant-bl-1
ChemiComp GT116	Competent <i>E. coli</i>	gt116-11
COVID-19 Product Range		
HEK-Blue™ hACE2 Cells	Cell line	hkb-hace2
A549-hACE2-TMPRSS2 Cells	Cell Line	a549-hace2-tpsa
pUNO1-hACE2	Expression vector	puno1-hace2
pUNO1-hTMPRSS2a	Expression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

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1 GGACCTGCAGGCCTGAAATAACCTCTGAAAGAGGAACTTGGTTAGGTACCTTCTGAGGCGGAAAGAACCAGCTGTGGAATGTGTGTAGTTAGGGTGTG
 101 GAAAGTCCCAGGCTCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCAGGCTCCCAGCAGGCAG
 201 AAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCACTAGTCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCAGA
 301 GAAGTTGGGGGGAGGGGTGCGCAATTGAACGGGTGCCTAGAGAAGGTGGCGGGGTAACAGTGGGAAAGTGTGCTGTACTGGCTCCGCCCTTTTCCC
 401 GAGGGTGGGGGAGAACCGTATATAAGTCAGTAGTCGCGGTGAACGTTCTTTTTCGAACGGGTTTGGCCGAGAACAGCTGAAGCTTCGAGGGGCTC
 501 GCATCTCTCTTACGCGCCCGCCCTACCTGAGGCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCTCCCGCTGTGGTGCCTCCTGAAGTGC
 601 GTCCGCCGCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGAGGCTACCTAGACTCAGCCGGCTCTCCACGCTTTC
 701 CTGACCTGCTTGTCAACTCTACGCTTTTGTTCGTTTTCTGTTCTGCGCAGTTACAGATCCAAGCTGTGACCGGCGCTACTCTGAGATCACCGGTCAA

Agel (791)

801 CATGTTTGTGTTCTTGGTGTGCTTCCACTGGTCAGTCCCAATGCGTAAATL18F (852) T20N (858) P26S (876)
 1▶ M F V F L V L L P L V S S Q C V N F T N R T Q L P S A Y T N S F T
 901 AGAGGAGTGTACTATCCTGACAAAGTGTTCGGTCAAGTGTCTCCACTTACTCAGGACCTTTTCTGCCTTTCTTTTCTAACGTTACATGGTTTCATG
 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 CAATCCATGTGCTGGGACAAACGGCACCAACGCTTCGACAACCTGTATTGCCATTCAATGATGGGGTGTACTTTGCCTCCACAGAGAAATCCAACAT
 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 CATTGAGGATGGATTTTGGGACTACTCTGGACTCAAAGACACAGAGCCTGCTGATCGTTAAACAACGCCACAAACGTTGTCATCAAAGTGTGCGAATTC
 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

D138Y (1212)

1201 CAGTTTTGCAATL138Y (1212) TATCCCTTCTGGGAGTGTACTATCACAAGAATAACAAGTCTGGATGGAGAGCGAATTTCCGGTCTACAGCAGCGCAAACAAGTGA
 134▶ Q F C N Y P F L G V Y Y H K N N K S W M E S E F R V Y S S A N N C

R190S (1368)

1301 CCTTCGAGTACGTGAGTCAACCTTTCTGATGGACCTGGAAGGAAACAGGAAACTTCAAGAACCTGAGTGTGTTGCTTTAAGAACATCGACGGCTA
 167▶ T F E Y V S Q P F L M D L E G K Q G N F K N L S E F V F K N I D G Y
 1401 TTTAAGATCTATAGTAAGCATACGCCATCAACCTGGTAAGGGATCTTCCCAGGGCTTTTCCAGCCTGGAACTTTGGTTGACTTGCTATTGGTATC
 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 AATATCACCAGATTTACAGACCTTCTGGCATTGCAICGGTCTTACTTACTCCAGTGATTCTCTCCGGTGGACTGCCGGCGCCGCTGCCTACTATG
 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 TCGGCTATCTGCAACCAAGAAGTCTCTGCTCAAGTACAACGAAACGGCACTATTACGGATGCTGTTGATTGTGCCCTGGACCCTCTGTCTGAGACTAA
 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 ATGCACCCTCAAGAGCTTTACCGTTGAGAAGGGGATTTACCAAAACAGTAATTTCCGGTCCAACCCACGAAAGCATTGTGCGGTTCCCAAATATACC
 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 AATCTGTGCCCTTTGGCGAAGTGTCAATGCTACAAGGTTTGTCTTGTGTACGCATGGAATAGGAAACGCATCTCCAATTGTGTCGCTGATTACTCCG
 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 TGCTGTACAATCCGCTCTTTCTCAACCTTCAAGTGTATGGCGTTTACCTACCAAACCTAACGACCTGTGCTTACTAATGTGTATGCCGACTCTTT
 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

K417T (2049)

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

2101 ATTCATGGAACCTCAACAATCTGGATTCCAAGTGGTGGCAACTATAACTACCTGTATAGACTGTTCCAGGAAATCCAACCTGAAACCTTCGAGCGAG
 434▶ I A W N S N N L D S K V G G N Y N Y L Y R L F R K S N L K P F E R

E484K (2250)

2201 ATATAAGCACAGAAATCTACCAGGCTGGAAGTACGCCCTGCAACGGCGTGAAAAGGGTTCAACTGCTACTTCCCATTGAGAGTTACGGATTCCAGCCTAC
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N501Y (2301)

2301 ATACGGGGTGGTTACCAACCTATCGTGTGCTAGTCTGAGTTTTGAGTCTCCATGCCCCAGCCACAGTCTGTGGCCCCAAGAAAAGCACAATCTG
 500▶ Y 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 GTGAAGAACAATGCGTGAACCTTAACTTTAACGGACTCACAGGAACCGGCGTATTGACGGAGAGTAACAAGAAGTCTGCCATTCCAGCAGTTCGGTC
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2501 GCGATATTGCCGACACTACCGACCTGTCCGAGATCCCAGACATTGGAGATTCTTGATATCACACCTGTAGTTTCCGGCGGAGTGAGCGTGATTACGCC
 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 CGGAACCAATACCAGCAATCAGGTTGCCGCTGTATCAGGGCGTGAATTGCACCGAGGTACCTGTCGCCATCCACGCTGACCAACTTACCCACATGG
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H655Y (2763)

2701 CGAGTATATTCCACCGCTCCAACGCTTTTCAGACAGCTGCTGGATGTCTGATCGGTGCAGAAACGTTAATAATAGCTACGAGTGTGATATCCCCATCG
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R685A (2853)

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667▶ G A G I C A S Y Q T Q T N S P R A A A S V A S Q S I I A Y T M S L G

R683A (2847)

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3001 ACAAGCGTTGACTGCACCATGTACATCTGTGGCGATTCTACCGAATGTAGCAATCTCTCCTGCAATACGGATCATTCTGCACTCAGCTGAATCGTGCCC
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3101 TCACAGTATTGCAAGTGTGAGCAGGACAAGAATACGCAAGTGTGTTGCCAGGTGAAGCAAACTACAAAACCTCCACCCATAAAAGACTTTGGCGGATT
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3201 CAATTTCTCACAGATCTGCCGATCCCTCAAACCTCCAAGCGTAGCTTTATCGAGGATCTGCTCTTCAACAAGGTAACCTCGCAGATGCCGGTTTC
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3301 ATCAAGCAGTATGGCGATTGTCTGGGAGACATCGCCGCTGGGACCTGATCTGTGCACAGAAGTTCATGGACTGACCGTGTGCTCCCTGCTGACCG
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3501 GCAGATGGCCTACCGCTTTAACGGCATCGGTGTGACACAAAACGTTCTGTATGAAAACGAGAACTCATCGCAACAGTTCAACAGTGCATCGGTAAG
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T1027I (3879)

3801 CCTTCAGTCTCTTCAGACCTATGTGACCAGCAGCTCATCCGCGTGTGAAATTCGCGCATCCGCTAACCTGGCAGCAATGAAAATGTCCGAGTGTGTG
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3901 CTGGGTGAGTCTAAGAGAGTGGACTTTTTCGCGGAAGGGGTATCACCTGATGTCTTTTCTCAGTCTGCACCCCATGGTGTGGTCTTTCTGCACGTGACTT
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4001 ATGTCAGCTCAGGAAAAGAACTTCACTACAGCCCAGCCATCTGCCACGATGGGAAAGCCACTTTCCAGGGAAGGCGTATTCGTGTCCAATGGTAC
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4101 TCATTGGTTGCTCACTCAGAGAAATTTCTACGAGCCCCAGATTATAACCACTGACAATACATTTGTATCCGGCAATTGTGATGTGGTTATCGGGATTGTG
1100▶ H W F V T Q R N F Y E P Q I I T T D N T F V S G N C D V V I G I V

4201 AATAACTGTTTACGATCCTTTGACGCCAGAGCTGGACTCCTTCAAGGAGGAGCTTGACAAATATTTAAGAATCACACATCACCTGACGTCGACCTCG
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V1176F (4326)

4301 GAGATATTTCAGGAATCAATGCTTCCCTTGTCAATATTCAGAAGGAGATAGACAGGCTGAATGAGGTTGCCAAGAACCTCAACGAGTCTCTGATCGATCT
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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 GAGTTTGACAAACCACAACCTAGAATGCAGTAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATA

4701 AACAAAGTTAACAAACAATTCATTCTTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAT

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5101 GAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGA

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