

# pUNO1His-SARS2-S2

Plasmid designed for the production of the SARS-CoV-2 Spike S2::His fusion protein

Catalog code: p1his-cov2-s2

<https://invivogen.com/sars2-spike-s2-tag-production-vectors>

For research use only

Version 21C17-ED

## PRODUCT INFORMATION

### Contents

- 20 µg of lyophilized pUNO1His-SARS2-S2 (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 stored at -20°C is stable at least for 1 year.
- Store Blasticidin at 4°C or -20°C. The expiry date is specified on the product label.

### Quality control

- After purification by ion exchange chromatography, predominant supercoiled conformation is verified by electrophoresis.
- Plasmid construct is confirmed by restriction analysis and full-length open reading frame (ORF) sequencing.

## PLASMID FEATURES

### SARS-CoV-2 Spike S2::His cassette

- **SV40 enhancer** is comprised of a 72-base-pair repeat and allows the enhancement of gene expression in a wide range of hosts. The enhancement varies from 2-fold in non-permissive cells to 20-fold in permissive cells. Furthermore, the SV40 enhancer is able to direct nuclear localization of plasmids<sup>1</sup>.
- **EF-1α/HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) core promoter<sup>2</sup> and the 5' untranslated region of the Human T-Cell Leukemia Virus (HTLV). EF-1α utilizes a type 2 promoter that encodes for a «house keeping» gene. It is expressed at high levels in all cell cycles and lower levels during G0 phase. The promoter is also non-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>3</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 possibly through mRNA stabilization.

- **Codon-optimized Spike S2 subunit ORF**

pUNO1His-SARS2-S2 contains the coding sequence for the S2 subunit of the Spike (S) protein from the original SARS-CoV-2 Wuhan-Hu-1 isolate. To improve expression of the S2 subunit, the gene is codon-optimized. Furthermore, to facilitate secretion of the protein the coding sequence is preceded by the native SARS-CoV-2 signal sequence. The S protein mediates the binding and entry of SARS-CoV-2 to host cells. In particular, the S2 subunit contains various features such as a fusion peptide and heptad repeats (HR), and is thus responsible for viral-host membrane fusion<sup>4</sup>.

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

- **His** is a polyhistidine tag cloned at the C-terminus of the gene of interest and followed by a Stop codon.

- **SV40 pAn** is the Simian Virus 40 late polyadenylation (pAn) signal enables efficient cleavage and polyadenylation reactions, resulting in high levels of steady-state mRNA<sup>5</sup>.

### Antibiotic selection cassette

- **hCMV (human cytomegalovirus) enhancer & promoter** drive the expression of the blasticidin resistance in mammalian cells.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **bsr (blasticidin resistance gene)** from *Bacillus cereus* encodes a deaminase that confers resistance to the antibiotic blasticidin. The *bsr* gene is driven by the CMV promoter/enhancer in tandem with the bacterial EM7 promoter. Therefore, blasticidin can be used to select stable mammalian cells transfectants and *E. coli* transformants.
- **Human beta-Globin pAn** is a strong polyadenylation (pAn) signal placed downstream of *bsr*. The use of beta-globin pAn minimizes interference and recombination events with the SV40 pAn signal<sup>6</sup>.

### General features of pUNO1His-SARS2-S2

- **pMB1 ori** is a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.

## 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.

## APPLICATIONS

### Detection and purification of the S2 subunit

pUNO1His-SARS2-S2 has been designed to generate the Spike protein S2 subunit in mammalian cells with a C-terminal polyhistidine (His) tag. This facilitates both the detection of the secreted protein with an anti-His antibody and its purification using an Ni<sup>2+</sup>-NTA column.

### Subcloning the gene into another vector

- For subcloning, two unique restriction sites flank the S2 subunit gene:
- **5' site:** AgeI (compatible with XmaI, BspEI, NgoMIV, and SgrAI)
  - **3' site:** NheI (compatible with XbaI, SpeI, and AvrII)

## TECHNICAL SUPPORT

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## REFERENCES

1. Dean DA. *et al.*, 1999. Sequence requirements for plasmid nuclear import. *Exp. Cell. Res.* 253:713-22. 2. 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. 3. 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. 4. Huang, Y. *et al.* 2020. Structural and functional properties of SARS-CoV-2 spike protein: potential antiviral drug development for COVID-19. *Acta Pharmacol Sin* 41, 1141-1149. 5. 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. 6. 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.

## RELATED PRODUCTS

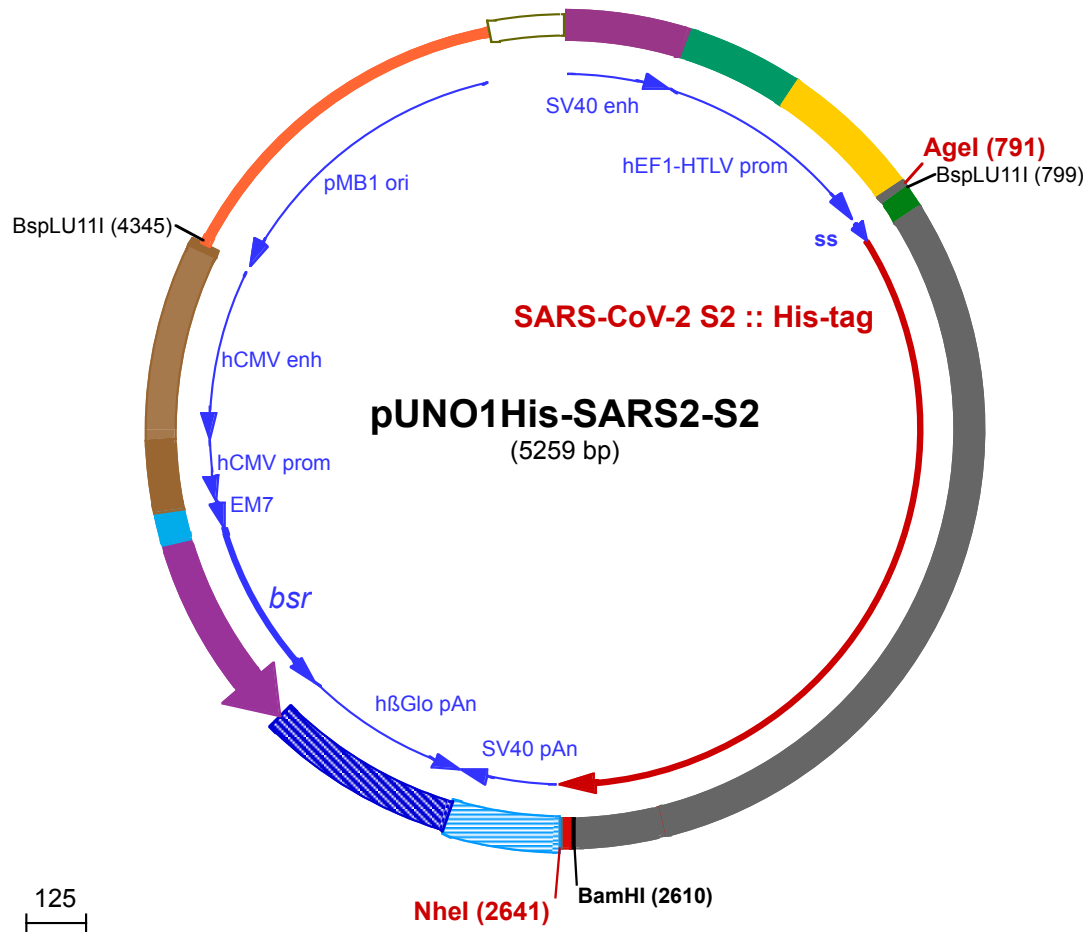
Product	Description	Cat. Code
Blasticidin	Selection antibiotic	ant-bl-1
ChemiComp GT116	Competent <i>E. coli</i>	gt116-11
<b>COVID-19 Product Range</b>		
HEK-Blue™ hACE2 Cells	Cell line	hkb-hace2
A549-hACE2-TMPRSS2 Cells	Cell Line	a549-hace2-tpsa
pUNO1-hACE2	Expression vector	puno1-hace2
pUNO1-hTMPRSS2a	Expression vector	puno1-htp2a
pUNO1-hTMPRSS2b	Expression vector	puno1-htp2b

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Agel (791)

1 GGACCTGCAGGGCTGAATAACCTCTGAAAGAGGAAGCTTGGTTAGGTACCTTCTGAGGCGGAAAGAACAGCTGTGGAATGTGTGTCAGTTAGGGTGTG  
101 GAAAGTCCCAGGCTCCCAGCAGGCAGAAATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAGTCCCAGGCTCCCAGCAGGCAG  
201 AAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCACTAGTCTCCGGTGGCCGTGAGTGGGAGAGCGCACATCGCCACAGTCCCCGA  
301 GAAGTTGGGGGAGGGGTGCGCAATTGAACGGGTGCCTAGAGAAGGTGGCGGGGTAACCTGGGAAAGTGTGCTGTACTGGCTCCGCTTTTTCCC  
401 GAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGGCCGAGAACAGCTGAAGCTTCGAGGGGCTC  
501 GCATCTCTCTTACGCGCCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCTCCCGCTGTGGTGCCTCCTGAACTGC  
601 GTCCGCGCTTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGC  
701 CTGACCCTGCTTCTCAACTCTACGTCTTTGTTTCTGTTTCTGTCGCGCTTACAGATCCAAGCTGTGACCGGGCGCTACCTGAGATCACCGGTCAA  
801 CATGTTTGTGTTCTTGGTGTGCTTCCACTGGTGGAGCTCCCAATGAGTGTAGCATCCAAAGTATCATTGCCTACACAATGAGCCTCGGTGCTGAGAAT  
1 M F V F L V L L P L V S S Q 1 S V A S Q S I I A Y T M S L G A E N  
901 TCTGTCGCTACAGCAACAACTCCATTGCTATCCCTACTAACTTCAATCAGTGTGACAAGTAAATCTGCCCGTATCTATGACCAAAAACAGCGTTG  
19 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 T S V  
1001 ACTGCACCATGTACATCTGTGGCGATTCTACCGAATGTAGCAATCTCCTCCTGCAATACGGATCATTCTGCACCTCAGTGAATCGTGCCTCACAGGTAT  
52 D C T M Y I C G D S T E C S N L L L Q Y G S F C T Q L N R A L T G I  
1101 TGCAGTTGAGCAGGACAAGAATACGCAGGAAGTGTTCGCCAGGTGAAGCAAATCTACAAAATCCACCCATAAAAAGACTTTGGCGGATTCAATTTCTCA  
85 A V E Q D K N T Q E V F A Q V K Q I Y K T P P I K D F G G F N F S  
1201 CAGATCTGCCGATCCCTCAAACCTCCAAGCGTAGCTTTATCGAGAGCTGTCTTCAACAAGGTAACCTCGCAGATGCCGGTTTCATCAAGCAGT  
119 Q I L P D P S K P S K R S F I E D L L F N K V T L A D A G F I K Q  
1301 ATGGCGATTGTCTGGGAGACATCGCCGCTCGGGACCTGATCTGTGCACAGAAGTCAATGGACTGACCGTGTGCCTCCCTTGTGACCGACGAGATGAT  
152 Y G D C L G D I A A R D L I C A Q K F N G L T V L P P L L T D E M I  
1401 AGCCCAATACACTAGCGCCTGTGGCCGGCACCATCACTTCTGGGTGGACATTCGGAGCTGGCGCTGCCCTTCAAGTTCCTTTTGTATGCAGATGGCC  
185 A Q Y T S A L L A G T I T S G W T F G A G A A L Q I P F A M Q M A  
1501 TACCGCTTAAACGGCATCGGTGTGACACAAAACGTTCTGTATGAAAACAGAAACTCATCGCAACAGGTTCAACAGTGTATCGGTAAGATACAGGATA  
219 Y R F N G I G V T Q N V L Y E N Q K L I A N Q F N S A I G K I Q D  
1601 GCCTGTCATCCACTGCCAGCGATTGGGAAAGTTGAGGATGTAGTGAACAGAAATGCCAGGCACTTAACACCCCTGGTGAACAGCTCTCTCAAATTT  
252 S L S S T A S A L G K L Q D V V N Q N A Q A L N T L V K Q L S S N F  
1701 TGGTGCCATTTCTAGCGTGTGAATGACATACTGAGCCGGTTGGACAAGGTGGAGGCTGAAGTGCAGATTGATAGGCTGATAACTGGGCGCCTTCAGTCT  
285 G A I S S V L N D I L S R L D K V E A E V Q I D R L I T G R L Q S  
1801 CTTAGACCTATGTGACCAGCAGCTCATCCGCGCTGTGAAATTCGCGCATCCGCTAACCTGGCAGCAACCAAAATGTCGAGTGTGTGCTGGGTGAGT  
319 L Q T Y V T Q Q L I R A A E I R A S A N L A A T K M S E C V L G Q  
1901 CTAAGAGAGTGGACTTTTGGGGAGGGGTATCACCTGATGCTTTTCTCAGTCTGCACCCATGGTGTGGTCTTTTGTGACGTTGACTTATGTCAGG  
352 S K R Y H A C T F C G K G Y H L M S F P Q S A P H G V V F L H V V F L Y V P A  
2001 TCAGGAAAAGAACTTCACTACAGCCCCAGCCATCGCCACGATGGGAAAGCCCACTTTCCAGGGAAGGCGTATTCTGTCCAATGGTACTTATGGTTC  
385 Q E K N F T T A P A I C H D G K A H F P R E G V F V S N G T H W F  
2101 GTCACCTCAGAGAAATTTCTACGAGCCCCAGATTATAACCACTGACAATACATTTGTATCCGGCAATTGTGATGTGGTTATCGGGATTGTGAATAACTG  
419 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 N N T  
2201 TTTACGATCCTTTGACGACGAGCTGGACTCCTTCAAGGAGGAGCTTGACAAAATTTTAAAGAAATCACACATCACCTGACGCTCGACCTCGGAGATATTC  
452 V Y D P L Q P E L D S F K E E L D K Y F K N H T S P D V D L G D I S  
2301 AGGAATCAATGCTTCCGTGGTCAATATTCAGAAGGAGATAGACAGGCTGAATGAGGTTGCCAAGAACCTCAACGAGTCTCTGATCGATCTGCAGGAGTTG  
485 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 Q E L  
2401 GGCAAGTACGAACAGTATATCAAATGGCCTTGGTACATTTGGCTTGGGTTTATTGCTGGGCTGATAGCTATCGTCATGGTGAACAATTATGTTGTGTTGCA  
519 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 L C C  
2501 TGACATCTGCTGTAGTTGTCTGAAGGGCTGCTGCTCATCGCGCAGCTGTTGCAAGTTTACGAGGACGATTCCGAGCCTGTGCTGAAGGGCGTCAAAT  
552 M T S C C S C L K G C C S C G S C C K F D E D D S E P V L K G V K L  
2601 GCATTATACGGGATCCGGCCATCATCATCACCATCACTAAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAAT  
585 H Y T G S G H H H H H H H •  
2701 GCAGTAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAAATTGCATT  
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3201 AATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTTAGTTCCTGGTGAT  
141 • N R T Y

3301 TTGAGGGGGATGAGTTCCTCAATGGTGGTTTTGACCAGCTTGCCATTCATCTCAATGAGCACAAAGCAGTCAGGAGCATAGTCAGAGATGAGCTCTCTGC  
135 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 A Y D S I L E R C

3401 ACATGCCACAGGGGTGACCACCCTGATGGATCTGCCACCTCATCAGAGTAGGGGTGCCTGACAGCCACAATGGTGTCAAAGTCCTTCTGCCGTTGCT  
102 M G C P S V V R I S R D V E D S Y P H R V A V I T D F D K Q G N S

3501 CACAGCAGACCCAATGGCAATGGCTTCAGCACAGACAGTGACCCTGCCAATGTAGGCCTCAATGTGGACAGCAGAGATGATCTCCCCAGTCTTGGTCTG  
69 V A S G I A I A E A C V T V R G I Y A E I H V A S I I E G T K T R

3601 ATGGCCCGCCGACATGGTGTGTTGTCTCATAGAGCATGGTGATCTTCTCAGTGGCGACCTCCACCAGCTCCAGATCCTGCTGAGAGATGTTGAAGG  
35 I A A G V H H K N D E Y L M T I K E T A V E V L E L D Q Q S I N F T

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2 K M

3801 TCTGACGGTTCACTAAACGAGCTCTGCTTATATAGACCTCCCACCGTACACGCCTACCGCCATTTGCGTCAATGGGGCGGAGTTGTTACGACATTTTGG

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4001 ATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAGGTCATGTACTGGGCATAAT

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4201 CACCCATTGACGTCAATGAAAGTCCCTATTGGCGTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGTCTTGGCGGTACGCCAGGC

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BspLU111 (4345)

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4601 ATAGCTCACGCTGATAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTACGCCGACCGCTGCGCTTATC

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