

pLV-SpikeV2

Expression vector for lentiviral pseudotyping with SARS-CoV-2 U.K. variant (B.1.1.7 lineage) Spike

Catalog code: plv-spike-v2

<https://www.invivogen.com/uk-b117-spike-pseudotyping-vector>

For research use only

Version 21D13-ED

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized pLV-SpikeV2 (plasmid DNA)

Storage and Stability

- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20°C.
- Resuspended DNA should be stored at -20°C and is stable for at least 1 year.

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

- **hCMV (human cytomegalovirus) enhancer & promoter** drives high expression of the SARS-CoV-2 spike gene in mammalian cells.
- **Rabbit (rbt) β-Globin intron** enhances the expression of the SARS-CoV-2 spike gene in mammalian cells.

- **Codon-optimized Spike ORF**

pLV-SpikeV2 contains the Spike coding sequence from the United Kingdom (U.K.) SARS-CoV-2 variant (B.1.1.7 lineage). This variant is characterized by a number of deletions (del) and mutations within the Spike coding sequence (see below)³. Additionally, to improve expression of the S protein in pseudovirions, the gene is codon-optimized and the last 19 amino acids, which contain an endoplasmic reticulum (ER)-retention motif (KxHxx), have been removed^{4,5}.

pLV-SpikeV2 includes the following sequence features:

- **S1 domain:** Del-H69-V70, Del-Y144, A570D, D614G, and P681H
- **RBD:** N501Y
- **S1/S2 boundary:** Functional furin cleavage site
- **S2 domain:** T716I, S982A, and D1118H

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 TMPRSS2³.

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

- **Rabbit β-Globin pAn** is a strong polyadenylation (pAn) signal placed downstream of the SARS-CoV-2 spike gene. It allows efficient transcription termination and polyadenylation of the mRNA.
- **bla (Ampicillin resistance gene)** encodes the β-lactamase enzyme, which confers resistance to the antibiotic ampicillin. Therefore, ampicillin can be used to select *E. coli* transformants.
- **pMB1 ori** is a minimal *E. coli* origin of replication.

APPLICATION

pLV-SpikeV2 has been designed for pseudotyping lentiviral particles with the SARS-CoV-2 Spike protein (U.K. variant). The basic strategy involves transfecting 293T cells with a lentiviral backbone plasmid encoding a fluorescent or luminescent reporter protein (e.g. GFP), a plasmid expressing the minimal set of lentiviral proteins necessary to assemble viral particles, and InvivoGen's pLV-SpikeV2. The transfected cells produce SARS-CoV-2 Spike-pseudotyped lentiviral particles, which can then be used to infect permissive cells.

GENERAL PROTOCOL

For a detailed protocol for producing SARS-CoV-2 Spike (S)-pseudotyped lentiviral particles, please refer to the literature⁴. In summary,

1. Co-transfect HEK293 cells with the plasmids required for lentiviral production. These include:

- **InvivoGen's pLV-SpikeV2** plasmid
- Lentiviral backbone plasmid encoding a reporter protein (e.g. GFP or Luciferase)
- Plasmid/s encoding the necessary virion packaging proteins

2. After ~48 hours, collect the S-pseudotyped lentiviral particles by harvesting and filtering the cell culture supernatant.

3. Determine the titre of the S-pseudotyped lentiviral particles using a permissive cell line that express the SARS-CoV-2 host receptor (e.g. InvivoGen's **HEK-Blue™ hACE2 cells**) in a relevant assay.

PLASMID PREPARATION

• 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.

REFERENCES

1. Shen, X. *et al.* 2021. SARS-CoV-2 variant B.1.1.7 is susceptible to neutralizing antibodies elicited by ancestral Spike vaccines. *Cell Host and Microbe*, doi: 10.1016/j.chom.2021.03.002. 2. Johnson, M.C. *et al.* 2020. Optimized Pseudotyping Conditions for the SARS-COV-2 Spike Glycoprotein. *J Virol* 94. 3. Hoffmann M. *et al.*, 2020. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell*. 181:1-16. 4. Crawford, K.H.D. *et al.* 2020. Protocol and Reagents for Pseudotyping Lentiviral Particles with SARS-CoV-2 Spike Protein for Neutralization Assays. *Viruses* 12. doi: 10.3390/v12050513.

TECHNICAL SUPPORT

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RELATED PRODUCTS

Product	Description	Cat. Code
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A549-hACE2-TMPRSS2 Cells	Cell Line	a549-hace2-tpsa
pUNO1-hACE2	Expression vector	puno1-hace2
pUNO1-hTMPRSS2a	Expression vector	puno1-htp2a
Anti-CoV2RBD-c1-hIgG1	Recombinant Antibody	cov2rbdc1-mab1

For a complete list of InvivoGen's COVID-19 related products visit:
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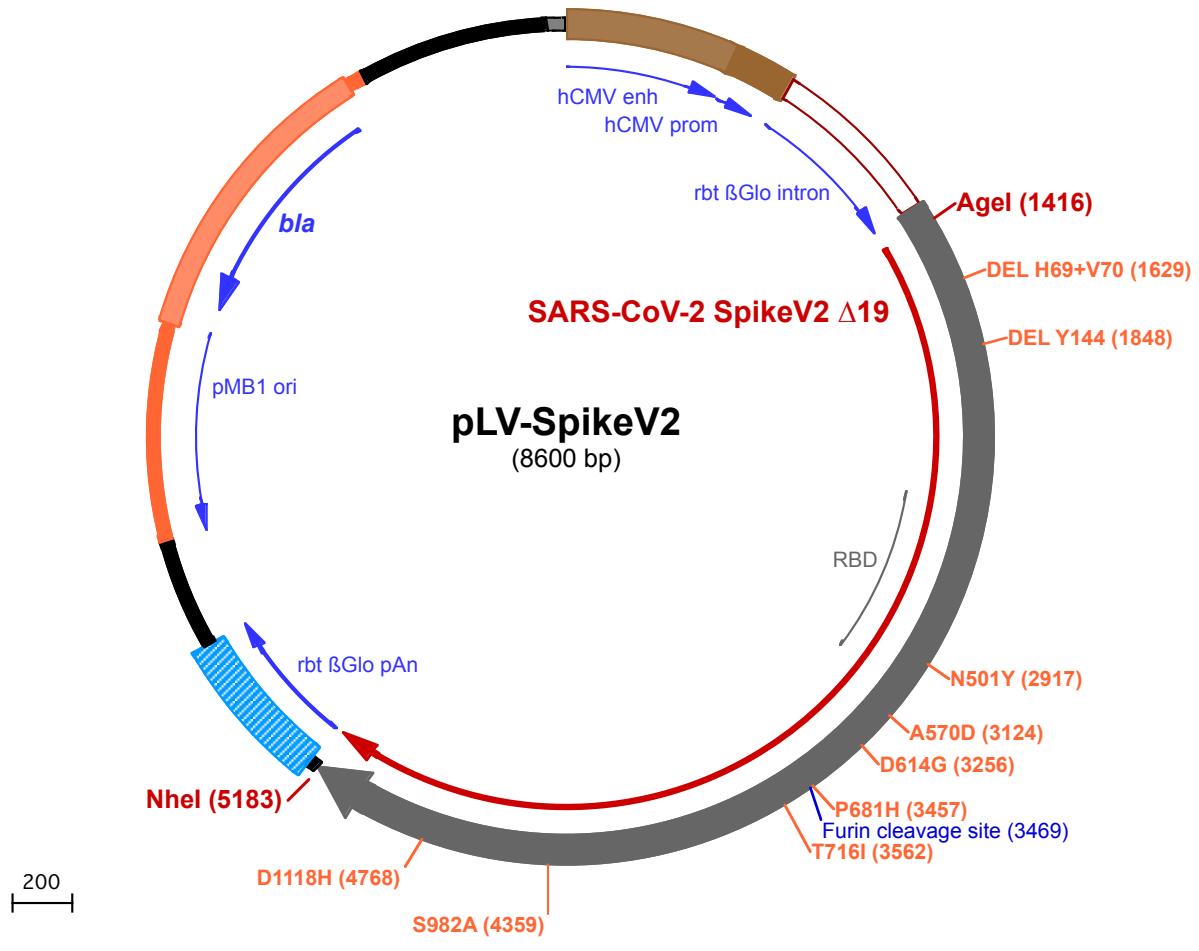
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1 GAGCTTGGCCATTGCATACGTTGTATCCATATCATAATATGTACATTTATATTGGCTCATGTCCAACATTACGCCATGTTGACATTGATTATTGACTA
101 GTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGGTTACATAACTACGGTAAATGGCCCGCTGGCTGACCGCC
201 CAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAA
301 ACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACA
401 TGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATA
501 GCGGTTTGACTCACGGGGATTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTGTTTGGCACAAAATCAACGGGACTTTCCAAAATGTCGTAAC
601 AACTCCGCCCATGACGCAAATGGGCGGTAGGCGGTACGGTGGGAGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCC
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1201 attggtagaacaactacaccctggtcatcatcctgcctttctctttatggttacaatgatatacactgtttgagatgaggataaaaactctgagtcca
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Agel (1416)

1401 ATTCTCGACGGATCCACCGTCAACATGTTTGTGTTCTTGGTGTGCTTCCACTGGTCAGTCCCAATGCGTTAATCTCACCCCGAACTCAACTCCC
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1501 ACCCGCATATACAAATTCCTTACCAGAGGAGTACTATCCTGACAAAGTTCGTTGCAAGTGTCTCCACTCTACTCAGGACCTCTTCTGCCTTTC
25▶ P A Y T N S F T 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
DEL H69+V70 (1629)
1601 TTTTCAACGTTACATGGTTTCATGCAATCTCTGGGACAAACGGCACAAACGCTTGCACAACCTGTATTGCCATTCAATGATGGGGTGTACTTTGCCT
59▶ F S N V T W F H A I S G T N G T K R F D N P V L P F N D G V Y F A
1701 CCACAGAGAAATCCAACATCATTGAGGATGGATTTTCCGGACTACTCTGGACTCAAAGACACAGAGCCTGCTGATCGTTAACACGCCACAAACGTTGT
92▶ S T E K S N I 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
DEL Y144 (1848)
1801 CATCAAAGTGTGCGAATTCAGTTTTGCAATGATCCCTTCTGGGAGTGTATCACAAGAATAACAAGTCTGGATGGAGAGCGAATTTCCGGTCTACAGC
125▶ I K V C E F Q F C N D P F L G V Y H K N N K S W M E S E F R V Y S
1901 AGCGCAAACAACGACCTTCGAGTACGTGAGTCAACCTTTCTGATGGACCTGGAAGGGAAACAGGGAAACTTCAAGAACCTGAGAGAGTTTGTCTTTA
159▶ S A N N C 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
2001 AGAACATCGACGGCTATTTAAGATCTATAGTAAGCATACGCCTATCAACCTGGTAAGGGATCTTCCCAGGGCTTTTCCAGCCTGGAACCTTTGGTTGA
192▶ K N I D G Y 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
2101 CTTGCCTATTGGTATCAATATACCAGATTTTACAGCCTTCTGGCATTGCAICGGTCTTATCTTACTCCAGGTGATTCTCTCCGGGTGGACTGCCGGC
225▶ L P I G I 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
2201 GCCGCTGCTACTATGTCGGCTATCTGCAACCAAGAAGCTTCTGCTCAAGTACAACGAAAACGGCACTATTACGGATGCTGTTGATTGTGCCCTGGACC
259▶ A A A Y Y 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
2301 CTCTGTCTGAGACTAAATGCACCCTCAAGAGCTTTACCGTTGAGAAGGGGATTTACCAAACAGTAATTTCCGGGTCCAACCCACCGAAAGCATTGTGCG
292▶ P L S E T K 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
2401 GTTCCCAAATATACCAATCTGTGTCCCTTTGGCGAAGTGTCAATGTACAAGTGTGCTTCTGTGTACGCATGGAATAGGAAACGCATCTCCAATTGT
325▶ F P N I T 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
2501 GTCGCTGATTACTCCGTGCTGTACAATTCGCCCTCTTTCTCAACCTTCAAGTGTATGGCGTTTACCTACCAAACCTTAAACGACCTGTGCTTCACTAATG
359▶ V A D Y S 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
2601 TGTATGCCGACTTTTTGTGATACGAGGCGATGAAGTGAACAGATTGACACAGAGTTCACACAGGGCAGACCGGCAAAATGCGGACTACAACCTACAAGTTCAGATGA
392▶ V Y A D S F 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
2701 CTTTACCGGATGTGTTATTGCATGGAACCTAAACAATCTGATTCCAAGTGGGTGGCAACTATAACTACCTGTATAGACTGTTCCAGGAAATCCAACCTG
425▶ F T G C V 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
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459▶ K P F E R 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

N501Y (2917)

2901 ACGGATTCCAGCCTACAATGGGGTGGGTTACCAACCCCTATCGTGTCTAGTCTGAGTTTTGAGCTCCTCCATGCCCCAGCCACAGTCTGTGGCCCAA
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3001 GAAAAGCACCAATCTGGTGAAGAACAATGCGTGAACCTTAACTTTAACGGACTCACAGGAACCGCGGTATTGACGGAGAGTAAACAAGATTCTGCCA
525▶ K S T N L 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

A570D (3124)

3101 TTCCAGCAGTTCGGTCGCGATATTGATGACACTACCGACGCTGTCCGAGATCCCCAGACATTGGAGATTCTTGATATCACACCCTGTAGTTTCGGCGGAG
559▶ F Q Q F G R D I D D T T D A V R D P Q T L E I L D I T P C S F G G

D614G (3256)

3201 TGAGCGTGATTACGCCCGAACCAATACAGCAATCAGGTTGCCGCTCTGTATCAGGGCTGAATTGCACCGAGGTACCTGTGCCATCCACGCTGACCA
592▶ V S V I T P G T N T S N Q V A V L Y Q G V N C T E V P V A I H A D Q
3301 ACTTACACCCACATGGCGAGTATATTCCACCGCTCCAACGTCTTTACAGACAGTGTGGATGTCTGATCGGTGCAGAACACGTTAATAATAGCTACGAG
625▶ L T P T W 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

P681H (3457) Furin cleavage site (3469)

3401 TGTGATATCCCCATCGGTGCTGGAATATGCGCCTTTATCAAACCTCAAACCAACTCTCACAGGCGGGCAGTAGTGTAGCATCCCAAAGTATCATTGCCT
659▶ C D I P I G A G I C A S Y Q T Q T N S H R R A R S V A S Q S I I A

T716I (3562)

3501 ACACAATGAGCCTCGGTGCTGAGAATTCTGTCGCCTACAGCAACAACCTCATTGCTATCCCTATTAACTTCAACAATCAGTGTGACAACTGAAATTCTGCC
692▶ Y T M S L G A E N S V A Y S N N S I A I P I N F T I S V T T E I L P
3601 CGTATCTATGACCAAAACAGCGTTGACTGCACCATGTACATCTGTGGGATTCTACCGAATGTAGCAATCTCCTCGTCAATACGGATCATTCTGCAT
725▶ V S M T K T S V 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
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759▶ Q L N R A L T G I A V E Q D K N T Q E V F A Q V K Q I Y K T P P I
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825▶ A D A G F I K Q 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
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925▶ S A I G K I Q D 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

S982A (4359)

4301 CTGGTGAACAGCTCTCTCAAATTTTGGTGCCATTTCTAGCGTGCTGAATGACATACTGGCAAGGTTGGACAAGGTGGAGGCTGAAGTGCAGATTGATA
959▶ L V K Q L S S N F G A I S S V L N D I L A R L D K V E A E V Q I D
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D1118H (4768)

4701 TCGTGCCAATGGTACTCATTGGTTCGTCACCTCAGAGAAATTTCTACGAGCCCCAGATTATAACCACTCACAAATACATTTGTATCCGGCAATTGTGATGT
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4801 GGTATCGGGATTGTGAATAACTGTTTACGATCCTTTGACCCAGAGCTGGACTCCTTCAAGGAGGAGCTTGACAAATATTTAAGAATCACACATCA
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1159▶ P D V D L 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
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1192▶ E S L I D L 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

NheI (5183)

5101 CATGGTGACAATTATGTTGTGTTGCATGACATCCTGCTGATGTTGTCTGAAGGGCTGTGCTCATGCGGCAGCTGTTGCTAAAGCTAGCCTCGAGGGATC
1225▶ M V T I M L C C M T S C C S C L K G C C S C G S C C •

5201 CGTCGAGGAATCACTCCTCAGGTGCAGGCTGCCTATCAGAAGGTGGTGGCTGGTGGCCAAATGCCCTGGCTCACAATACCACTGAGATCTTTTCCC

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263 G T T Y I V V I R S P K G D P G L A A I I G R S G R E G A G S K D
7101 AGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGAAGCTAGAGTA
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